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Pathways of fetal and early neonatal infection

Viral placentitis, bacterial and fungal chorioamnionitis

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IN RECENT years pediatricians, obstetricians, and pathologists have shown a renewal of interest in antenatal events and in environmental factors affecting fetal and neonatal health. Such factors are the infections of the placenta or of the amniotic fluid and have been known for many years.[1-6] The idea that most bacterial infections of the neonate are acquired after birth is, however, still prevalent. Bacterial infection of the amniotic cavity and the risk of its extension to the fetal organism are usually considered of minor practical interest. This attitude might explain the present status of the subject of prophylaxis and treatment of amniotic infection in the mother and in the child. These are still unsolved problems.

The diagnosis of infection in the neonatal period presents considerable difficulties,[7-10] and the prophylactic administration of antibiotics to infants carries dangers which should be weighed against the actual risks of infection.[11] Objective criteria of intrauterine exposure to infection[12-16] may be derived from our knowledge of the pathogenesis of prenatal infection and might help to screen the "high risk" babies. Such tests might prove of more than academic value, if and when effective and safe antibiotics become available for treatment of the infected full-term or premature newborn

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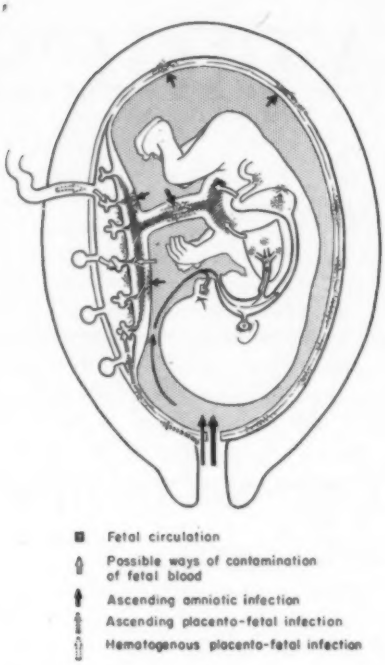


Fig. 1. Routes of fetal infection.

infant. As of now they constitute a basis for presumptive diagnosis of neonatal infection.

ROUTES OF INFECTION[17-19]

There is a basic difference between antenatal and intranatal infection. Practically all instances of antenatal infection of the fetus result from hematogenous dissemination of viruses, bacteria, fungi, or protozoa. The microorganism is carried to the intervillous spaces of the placenta by the maternal circulation and penetrates into the fetal blood stream.

In contrast, intrapartum infection is much more common, and is due to the ascension of vaginal bacteria into the amniotic cavity, usually after rupture of the membranes. The transmission of infection to the fetus takes place essentially through the mouth and nose (orificial infection), and the most common and severe lesions are found in the lungs. The inflammatory lesions observed in such cases in the membranes, umbilical cord, placenta, and fetus are conveniently grouped under the name of "amniotic infection syndrome." Fetal sepsis might in turn result from amniotic infection, either after passage of bacteria through the mucosa of the gastrointestinal[20] or respiratory tracts, or after direct invasion of the chorionic vessels on the placental surface (Fig. 1).[6]

Another type of hematogenous dissemination from placenta to fetus may take place in some instances of ascending infection with deciduitis (ascending placentofetal infection[18]). Infection of the amniotic fluid through the Fallopian tube has been shown to occur[17] but appears of little practical importance.

Hematogenous placentofetal infections result from maternal sepsis which may be due to a great variety of microorganisms. Ascending infections, amniotic and placentofetal, are usually due to vaginal bacteria. Ascending placentofetal infection is usually combined with amniotic infection,[18] whereas the latter is generally isolated. Simultaneous bacterial invasion of both maternal and fetal tissues is to be expected in ascending placentofetal infection, but appears to be uncommon in amniotic infection.[20]

Transplacental infection is less common than bacterial amniotic infection and probably of much less practical importance. This review will deal essentially with the "amniotic infection syndrome." The interest of placental examination in perinatal infection will be illustrated by two unusual cases; one, a fungal amniotic infection due to *Candida albicans*, the other, a viral placentitis in cytomegalic inclusion disease.

HEMATOGENOUS PLACENTOFETAL INFECTION

These infections occur at any time in the antenatal period. Some affect the embryo while others tend to affect the fetus late in pregnancy (toxoplasmosis, cytomegalic inclusion disease, Coxsackie and Listeria infections). Such conditions actually belong to the field of prenatal pediatrics, and some may be prevented by adequate measures.[9]

An extensive review of the subject is made in Flamm's monograph[19] and in recent articles.[9, 12]

Etiology. Infectious agents said to produce transplacental infection of the mammalian fetus include: viruses (those causing rubella, herpes simplex, herpes zoster, variola, varicella, poliomyelitis, measles, various encephalitides, cytomegalic inclusion disease, hepatitis, mumps, mononucleosis, lymphogranuloma inguinale, influenza, psittacosis, and the Coxsackie and Rickettsia and some as yet unidentified viruses[10]); bacteria (enteric bacteria, staphylococci, streptococci, pneumococci, *Vibrio cholerae* and *V. fetus*, Clostridium, Pasteurella, *Listeria monocytogenes*); species of Mycobacterium (*M. tuberculosis, M. lepra*); spirochetes *Borrelia recurrentis, Treponema pallidum* and *T. pertenue,* Leptospira); fungi (Histoplasma, Coccidiomyces, Candida); protozoa (Plasmodium, Trypanosoma); and even worms.

For a number of these organisms, the possibility of fetal infection has been demonstrated only in the experimental animal. The list of infections of pediatric interest is much shorter. Among viruses, one may mention those producing early embryonal damage (embryopathies), such as rubella and possibly poliomyelitis and influenza,[22] or late damage in the fetus or in the newborn, such as Coxsackie, poliomyelitis, and cytomegalic inclusion disease.

Bacteria can rarely be incriminated in transplacental infection. Examples of fetal or neonatal contamination related to maternal sepsis include infection by *Escherichia coli,* staphylococci, streptococci, pneumococci,[7, 18, 19, 23] and *Listeria monocytogenes.* Congenital tuberculosis and, nowadays, syphilis are quite rare.

Toxoplasma is the only known protozoon responsible for relatively frequent transplacental infection.

Pathogenesis and placental pathology. The exact mechanism of transplacental passage has not been elucidated and it is not known whether or not placental lesions are a prerequisite for the passage of microorganisms. More studies of the placentas of infants

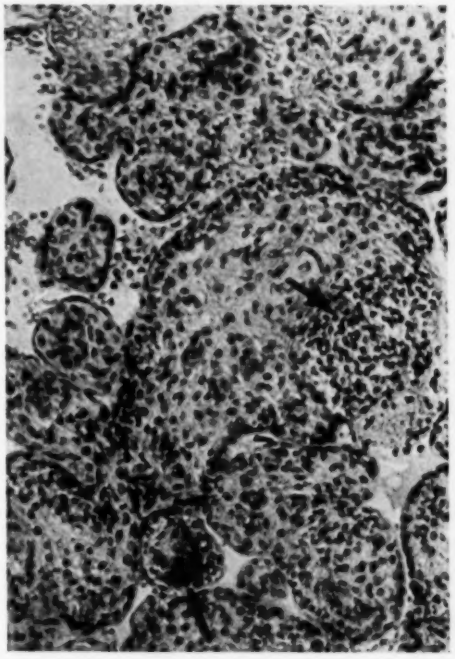


Fig. 2. Placental sepsis in late abortion. Clump of bacteria in vessel of villus (upper arrow) and inflammatory infiltrate (lower arrow). (Hematoxylin and eosin. ×200; reduced ⅙.)

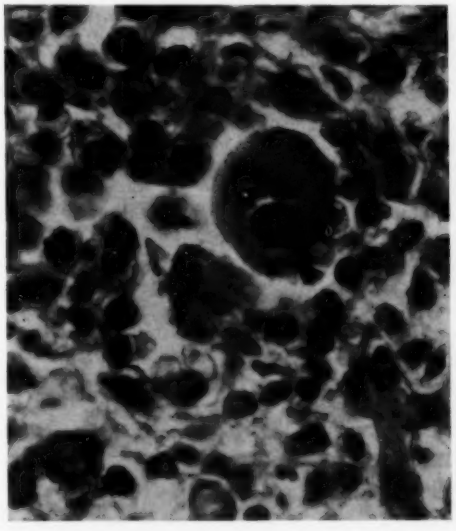


Fig. 3. Cytomegalic cell in pulmonary alveolus. (Hematoxylin and eosin. ×900.)

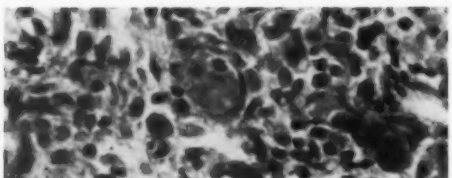


Fig. 4. Cytomegalic cells in pulmonary capillaries. (Hematoxylin and eosin. ×900; reduced ½.)

born during a septic phase of a maternal disease might provide further information on this point. No changes were found in placentas of cases of maternal brucellosis, coccidioidomycosis, and schistosomiasis examined in this laboratory.

In human beings, alterations in the placenta have been found in a few instances of hematogenous infection and resembled, histologically, the lesions produced by the same agents in other organs. The changes are initially localized in the intervillous space and spread to the adjacent villi. In coccidioidomycosis[24] the placenta may be severely involved while the fetus remains free of infection. Intervillositis, the inflammation of the intervillous spaces, has been observed in association with fetal bacteremia or fetal lesions in tularemia,[25] vibrio infection,[26] gas bacillus infection,[27] listeriosis,[28] leprosy,[29] tuberculosis,[30] and toxoplasmosis.[31]

An interesting mechanism of infection has been observed in a few cases of congenital tuberculosis. The placentas were infected by hematogenous dissemination, and tubercles developed at the surface of the chorion with secondary contamination of the amniotic fluid and orificial infection of the fetuses.[30]

Syphilis is said to produce typical placental changes. This specificity is doubted by experts.[32] The changes formerly described as syphilitic are probably secondary to maceration or to unrelated conditions, such as erythroblastosis, which were not known years ago.

Placental sepsis. Septic foci in the villi are due to hematogenous dissemination of microorganisms in fetal sepsis.[5] In its usual form it is a part of fetal sepsis following the severe amniotic infection seen in septic abortion (Fig. 2). The extensive placental lesions of listeriosis develop in the same way. The mother usually has a mild form of the disease during which transplacental contamination of the fetus takes place through an intact placenta. The bacteria develop rapidly in the fetal blood, are embolized in the pla-

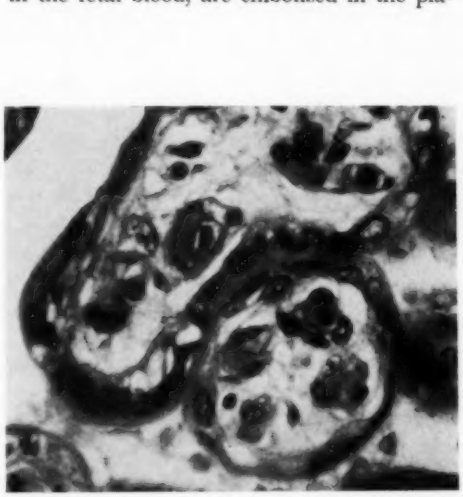


Fig. 5. Cytomegalic cell in placental villus, probably an endothelial cell. (Hematoxylin and eosin. ×900; reduced ⅙.)

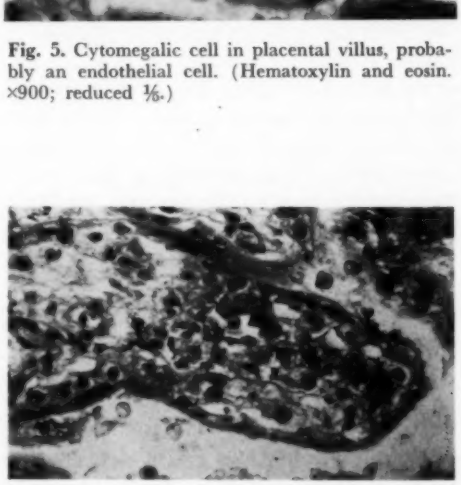


Fig. 6. Acute inflammatory reaction and necrosis in placental villus around degenerated cytomegalic cell. (Hematoxylin and eosin. ×600; reduced ½.)

centa in large numbers, and produce extensive lesions which are, in turn, the source of massive maternal reinfection.[19] Listerial chorioamnionitis might be primary or secondary to infection by bacteria passed with fetal urine.

Very little is known about the placenta in virus infections and it is assumed that the viruses cross intact chorionic villi. The placentas of one case of congenital poliomyelitis and of three parturients with poliomyelitis were normal,[33] as was that of a case of congenital Coxsackie infection.[34] Necrotic foci identical with those seen in other tissues are said to occur in the placentas of women with varicella and smallpox.[35] Very similar lesions have been described in a case of prenatal infection with vaccinia.[36] LeTan Vinh[37] has seen cytomegalic inclusion cells in the placenta of a case of neonatal cytomegalic inclusion disease[38] and mentions an identical finding in another case. There was in addition moderate plasma cell infiltration of some villi.[38] This case is as yet unique. A similar case was observed in Sloane Hospital and will illustrate some of the difficulties of interpretation.

Transplacental infection with cytomegalic inclusion disease and viral placentitis. A premature, nonmacerated stillborn infant was born during the twenty-fifth week of gestation after an 11-hour labor. At autopsy, a typical amniotic infection syndrome was diagnosed. There was severe placentitis and congenital pneumonia due to a gram-negative bacterium. This was not suspected grossly, and no cultures were taken. There were petechiae on the skin and on the thoracic and abdominal serosa. Numerous typical cytomegalic cells, frequently undergoing necrosis, were found in the capillaries of all organs. Some were located within endothelial cells whereas others were apparently in the circulating blood. A number of inclusion-bearing cells were lying in pulmonary alveoli; but only a few were seen in the kidneys. There was no evidence anywhere of granulomatous or interstitial inflammation except in the brain which showed rare small epitheloid nodules. In the placenta a number of typical cells were identified in the villi, both in capillaries and in large chorionic histiocytes. In many areas this was accompanied by acute focal necrosis and by degeneration of the few adjacent monocytic and neutrophilic inflammatory cells (Figs. 3-6). There were no bacteria in these foci, so that it is improbable that necrosis of the villi was secondary to fetal sepsis resulting from severe amniotic infection. No inclusion cells were found in the trophoblastic cells.

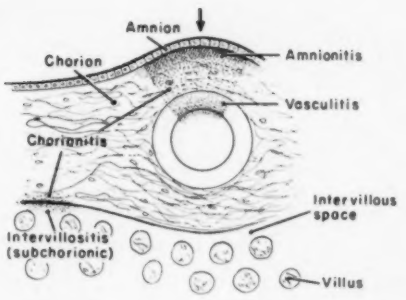


Fig. 7. Schematic representation of the inflammatory response (small dots) of the placenta to bacterial infection of the amniotic cavity (arrow).

Early manifestations of viral infection were observed in this case, and the possibility that the virus might be transported in the circulation within infected cells is strongly suggested by the morphologic data. The placental lesions differ from those described in the cases of Lepage and Schramm[38] and Lelong and co-workers.[37] Necrosis is not uncommon in other viral diseases. It is not a striking feature of neonatal cytomegalic inclusion disease in which inflammatory changes are usually evident. In the present case, inflammation was lacking or minimal in all organs. The lack of cellular reaction is not due to inadequate fetal defense mechanisms because there was a marked neutrophilic response to bacterial amniotic infection. Thus, these unusual placental lesions may represent an early stage of the disease. Plasmocytic infiltrates[38] probably formed by modified Hofbauer cells[39] might appear only in the later stages. Plasma cells are not de-

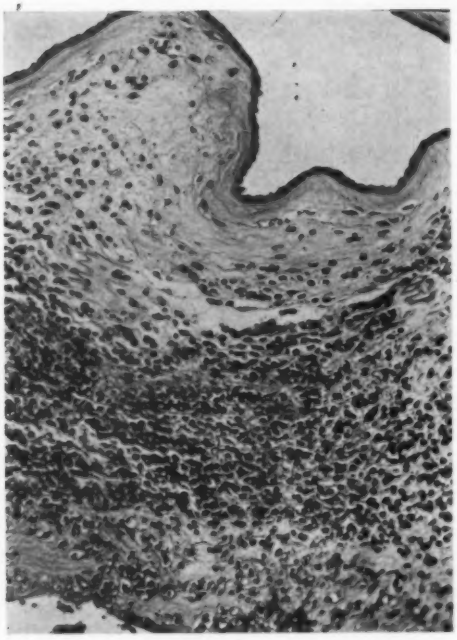


Fig. 8. Membranitis. The neutrophils spread from the decidua (bottom) toward the amnion (top). (Hematoxylin and eosin. Original magnification ×240; reduced ⅕.)

scribed in the placenta of the case of vaccinia,[36] although they were abundant in the visceral infiltrates. It has been suggested that the observation of more severe changes in the placenta than in other organs points to the lesions of the chorionic villi as the portals of entry.[36] It is, however, quite possible that both fetal and placental alterations are part of the same phase of viral dissemination. Direct inoculation of cytomegalic inclusion virus[40] in the placentas of guinea pig fetuses resulted in local lesions similar to those obtained when the virus was injected in the fetal brain.

Pathologic conditions in the fetus. A detailed description of the pathologic anatomy of the embryo, the fetus, and the newborn in transplacental infection is beyond the scope of this article, and only some general principles and examples will be mentioned.

The type, distribution, and effects of lesions vary with the microorganism and the timing of infection. Rubella typically affects the embryonic development, but some late infections of the fetus also result in malformations (e.g., schizogyria in cytomegalic inclusion disease[41]). The incidence of viral or bacterial infection resulting in embryonic or fetal death is not known. The indirect role of maternal infection has been well substantiated in pyelonephritis, and the association of premature labor with bacteremia is attributed to the effect of the bacterial endotoxin on the uterus.[42]

The pathologic lesions of congenital tuberculosis provide an example of bacterial infection of the fetus. Mycobacteria carried through the umbilical vein produce tuberculous lesions predominantly in the liver. However, if the amniotic fluid is contaminated, orificial infection occurs and primary tuberculous foci may be found in the lungs, the intestinal tract, or even the middle ear of the newborn infant.[30, 43, 44] A similar type of contamination has been reproduced in experimental moniliasis of pregnant mice.[45]

In most viral and bacterial lesions, the pattern of visceral involvement does not clearly trace the pathways of invasion and depends on the tropism of the germ for certain tissues.

The incidence of lethal hematogenous infection of the fetus in the perinatal period is very low. The morphology of the occasional instance of septicemia due to a pyogenic organism is not specific. The pathology of syphilis, tuberculosis, cytomegalic inclusion disease, and toxoplasmosis is well known.

Table I. Relationship between the time elapsed from rupture of the membranes to delivery and the finding of bacteria in the amniotic fluid collected by means of transabdominal puncture (from Gosselin[20])

Time (in hours)	*No. of cases*	*Bacteria isolated from amniotic fluid*	
		No. of cases	*%*
0-6	11	1	9
6-10	14	4	29
10-24	15	7	47
24+	23	17	74

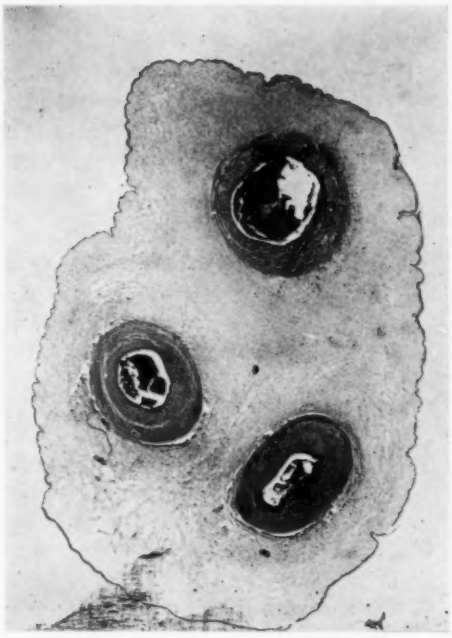


Fig. 9. Moderate funisitis with involvement of the umbilical vein (top) and of one artery (bottom right). The arrows point to the dark crescent formed by the infiltration of polymorphonuclear leukocytes migrating toward the amnion. (Hematoxylin and eosin. ×10; reduced ⅓.)

Small foci of coagulation necrosis with mild inflammatory reactions are found in the liver and adrenals in Coxsackie infection along with myocarditis and encephalomyelitis.[46] In listeriosis, the septic form is accompanied by multiple miliary foci of necrosis with cellular infiltration into the skin, esophagus, rectum, leptomeninges, and all viscera, particularly the liver, adrenals, spleen, and lungs. Many gram-positive rods can be found in the lesions. A number of the infants are stillborn. Chorioamnionitis due to Listeria may be present.[47]

ASCENDING PLACENTOFETAL INFECTION

Acute inflammation and microabscesses of the decidua and intervillous spaces result from infection by vaginal bacteria, usually in abortions. The bacteria might spread to the fetal circulation and produce fetal sepsis. In Sorba's series, deciduitis, subdecidual intervillositis, and microabscesses were found in 4 of 74 placentas. Three of the 4 fetuses presented metastatic septic lesions (pulmonary abscess, adrenal abscess, and purulent pericarditis).

This route of infection is seldom clearly demonstrated except in abortions and small premature infants. It is usually combined with amniotic infections and the same organism probably causes both types of infection.

AMNIOTIC INFECTION SYNDROME

The fetal adnexa.

Factors favoring amniotic infection. Factors which enhance the penetration of germs from the vagina are: premature rupture of the fetal membranes with long delay before the onset of labor, prolonged labor even with late rupture of the membranes, a prolonged period of dilatation even with intact membranes, and excessive manipulation. Obviously, germs from the outside may be introduced by contaminated hands or instruments. During rectal examination the vaginal content is "milked" into contact with the cervix and might also contaminate the membranes or the fluid as readily as when vaginal examination is performed. Infection may take place through intact membranes,[48] but this appears to be rare.[40] It is likely, but not demonstrated, that, when chorioamnionitis is found in association with clinically intact membranes, there was actually a high or inapparent rupture of the membranes.

Table II. Relationship between duration of labor after rupture of the membranes and infection of the amniotic fluid (from Gosselin[20])

Time (in hours)	*No. of cases*	*Bacteria isolated from amniotic fluid*	
		No. of cases	*%*
0-6	23	1	4
6-24	21	11	52
24+	19	17	89

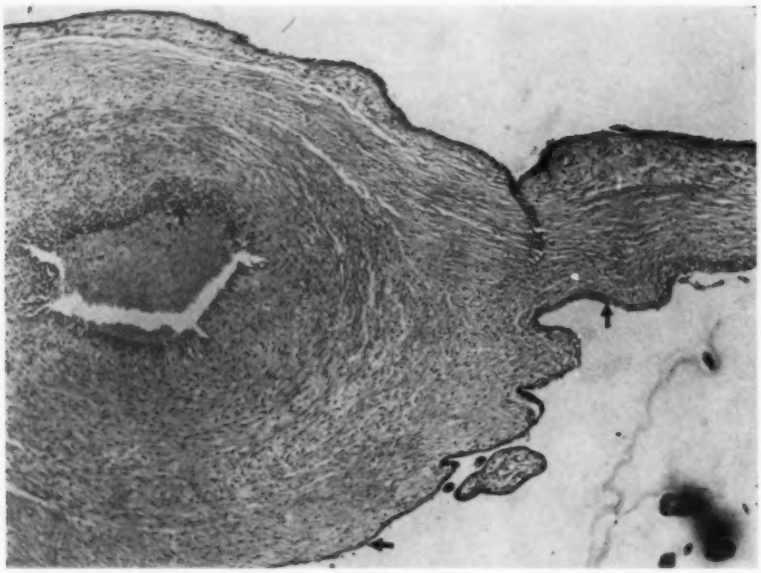


Fig. 10. Section of fetal placental surface. The amniotic cavity is at the top of the picture. The fetal reaction is manifested (arrow on the left) by infiltration of the vascular wall facing the amnion. There is only a moderate sprinkling of neutrophils in the chorion and immediately underneath the amnion. The maternal reaction—subchorionic intervillositis—is slight (arrow on the right) with minimal chorionitis. There is no maternal reaction where the chorion is thick (arrow at bottom of picture). (Hematoxylin and eosin. ×100; reduced ¼.)

The role of premature rupture of the membranes and of prolonged labor in the development of amniotic infection is well established.[3, 4, 12, 14, 49, 50] An even more important factor is the duration of active labor after rupture of the membranes (Tables I and II).

In one series, the membranes ruptured prematurely in 35 per cent of the cases with intact placentas, and the percentage increased only to 50 per cent of the cases with placental inflammation.[15] The mean lapse of time after the rupture, however, was 5 hours and 40 minutes in the intact placenta group as opposed to 12 hours and 15 minutes in the group with placentitis. For McIlwaine, the occurrence of placentitis rose from 3.3 per cent in the first 6 hours after the rupture to 16.3 per cent between 6 and 24 hours, and to 51.7 per cent in those over 24 hours.[51]

There is no uniformity of opinion concerning the time to be set as the danger line. The time limit chosen to analyze the incidence of infection or neonatal mortality in two groups born before and after this time varies from 9 hours[16] to 17 hours[49] or to 24 hours.[52] It is clear from the best available study[20] that the risk of exposure to bacteria increases sharply after 6 hours of labor with ruptured membranes.

Another factor favoring infection is prematurity. It was shown that placental inflammation is 3 times as common in premature births as in term deliveries,[15] a fact possibly related to the increased incidence of prolonged latent periods in premature labor.[50]

Finally, there is a higher incidence of infection in cases of uterine inertia with high forceps extraction[53] and when the stillborn infants are heavier.[54]

Bacteriology. The most common bacteria found in the amniotic fluid[20] or in the cord

blood[6] are *E. coli, Streptococcus faecalis,* and *Staphylococcus albus* and *S. aureus* (hemolytic and nonhemolytic). Similar findings were reported in a study of high vaginal swabs in puerperal pyrexia.[55] The bacteria found by Gosselin[20] in the amniotic fluid were always present in the vagina as well. He also found bacteria in the heart blood of 17 of 114 stillborn infants. These bacteria had been identified previously in their amniotic fluid. A strain of *S. aureus* has been found both in the maternal vagina and in the lungs of infants with neonatal pneumonia.[56] The same group of organisms which are commonly encountered in the perianal area and in the birth canal, are also grown from cultures of fetal or neonatal blood or tissues particularly in cases of perinatal pneumonia or septicemia.[57-62]

Other microorganisms such as *M. tuberculosis, Listeria monocytogenes,* or *Candida albicans* are rarely found. No simultaneous study of large series of cases of the bacterial flora in the vagina, in amniotic fluid, and in the neonatal blood or neonatal tissues at autopsy is available. There is considerable circumstantial evidence, however, which suggests that the "ordinary" bacteria of the birth canal simultaneously contaminate both the amniotic cavity and the fetus.

The bacterial origin of amniotic inflammation was demonstrated thirty years ago by Wohlwill and Bock. When the amniotic fluid is sterile and free of neutrophils, the placenta shows no inflammation.[15] The number of bacteria found in neonatal gastric smears roughly parallels the number of inflammatory cells.[15] In diamniotic twin deliveries, only one amniotic cavity is inflamed, as a rule, and it is the cavity which ruptured first.[15, 16, 18] Experimentally, the whole amniotic infection syndrome has been produced in animals.[63]

Other suggested causes of amniotic inflammation. It has been suggested that amniotic inflammation was too frequent to be due to bacterial infection and was related to changes in pH of the amniotic fluid secondary to the activity of the uterine muscle.[64, 65] This has never been substantiated. The pH changes were not found to correlate with infection or inflammation, whereas there was a close relation between inflammation and the presence of bacteria. A chemical chorioamnionitis may, however, occur in special circumstances such as the injection of an abortifacient into the amniotic cavity. Hypertonic saline solution, injected aseptically, produces an acute chorioamnionitis in human beings.[15] Meconium is said to induce such changes in rare instances,[66] but this has not been confirmed.

Recently, leukocytic infiltration of the umbilical cord has been found to be significantly associated with long labor, meconium staining, and positional abnormalities of the cord,[67] and, thus, attributed to fetal anoxia. No search for bacteria has been reported. The association with long labor may well be due to an increased incidence of infection; the same is true of a prolapsed cord. The mortality rate of infants born after the above complication was not higher, however, than that of newborn babies by normal delivery. One would expect a higher mortality rate in the group suffering from an asphyxia severe enough to produce vascular inflammation.[68] Furthermore, the mechanism of the action of asphyxia or leukopedesis is not clear. The leukocytes always migrate toward the amnion and are more abundant in the segments of vessels located closer to the amnion. This implies that the leukotactic

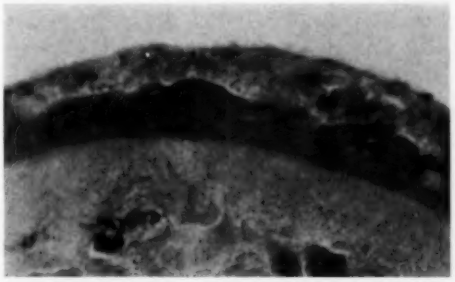


Fig. 11. Bacteria on the surface of the amniotic epithelium in a case of amniotic infection. The epithelium is necrotic in places, a not uncommon event. It is covered with amorphous debris containing plumb rods. (Hematoxylin and eosin. ×900.)

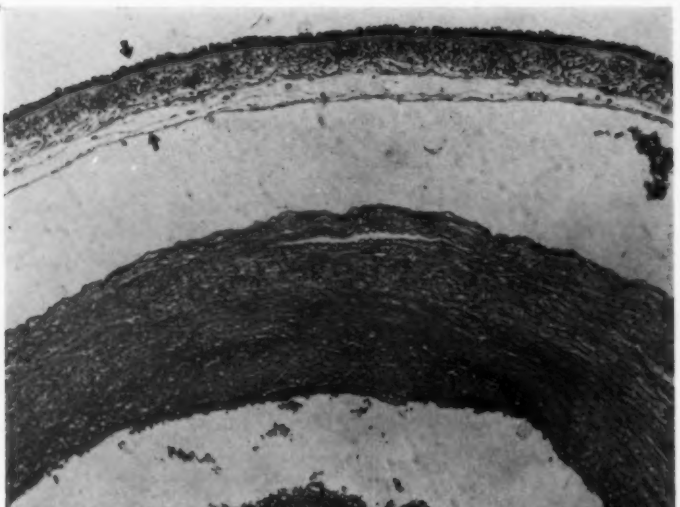


Fig. 12. Phlebitis and chorioamnionitis. The amnion and part of the upper chorion (between the arrows) became artificially separated from the chorion. It is such an area that is used for the whole mount technique (see Fig. 23). (Hematoxylin and eosin. ×100; reduced ¼.)

factor is present in the amniotic cavity and not in the fetal blood. It has not been shown that fetal asphyxia produces chemical changes of the amniotic fluid which are capable of inducing fetal leukopedesis. At the present time the evidence in support of a nonbacterial origin of chorioamnionitis is, scant, at best.

Pathology. The pathologic condition of the inflamed placenta has been described in old[5, 69] and recent[15, 18] studies. The lesions are illustrated in Figs. 7 to 13. The migration of polymorphonuclear leukocytes is apparently elicited by a factor present in the amniotic cavity or at the surface of the amnion and is always directed towards the amniotic epithelium. The infiltrates appear first in the membranes, particularly at the lower pole of the amniotic sac and stem from maternal vessels in the decidua. The earliest inflammatory infiltrate in the placenta is also made up of maternal neutrophils which are collected in the subchorionic intervillous space and which subsequently infiltrate the chorion. In small abortuses (below 20 cm. crown-heel) there is no neutrophilic response, and the infiltrates are usually purely maternal. The chorionitis may be extensive, but there is no leukopedesis through the fetal vessels in the chorion. A similar situation is encountered if amniotic infection occurs after fetal death. In small fetuses, maternal leukocytes appear to participate in fetal defense, within the lungs for example.[70, 71] Besides the participation of the cells of one organism in the inflammatory response within another, amniotic infection presents another unique aspect of inflammation.[15] In most inflammations, leukopedesis takes place at the capillary level. Since there are no capillaries in the chorionic plate or in the umbilical cord, leukocytic infiltration occurs through the walls of large vessels.

The fetal reaction usually starts in the vessels whose walls are relatively thin and only in that segment of the wall which abuts on the amniotic surface. It may be severe

enough to produce an acute arteritis or phlebitis with thrombosis. In the cord, the vein is usually the first to be inflamed.

Finally, the chorion shows massive infiltration and the neutrophils pass into the amniotic fluid. In chorioamnionitis, these cells accumulate in large numbers under the amnion.

Two main factors seem to govern the inflammatory response. One is the thickness of the barrier between the fetal or maternal vascular spaces and the amniotic surface. The thicker the barrier, the less is the reaction. Another factor is the nature of the leukocytes. Since subchorionic intervillositis is much more common than the fetal reaction, one might assume a different reaction threshold for fetal and maternal neutrophils. Inflammation of the membranes is frequently isolated, but, as a rule, chorioamnionitis and funisitis (the inflammation of the cord) are associated. In the cord, inflammation is usually segmental. It is probable, but not proved, that inflammation of the cord is more common in the segment located closer to the cervix. Isolated funisitis is found in cord prolapse.

Large chorionic histiocytes (Hofbauer cells) are frequently numerous in amniotic infection and are part of the tissue response. In the cases of amniotic infection associated with meconium staining of the placenta, these cells engulf meconium droplets (meconium histiocytosis). The finding of these cells and their presence close to the amnion or deep in the chorion has been used as an indication of the timing of meconium release by the fetus.

Incidence. The incidence of inflammatory changes in the chorion is 17 to 19 per cent and in the cord 11 per cent of all placentas, including the earliest stages of margination of neutrophils along the chorion in the intervillous space, and in chorionic and umbilical vessels. Chorioamnionitis is seen in 7 to 15 per cent of all placentas and is found, in association with funisitis, in 7.5 per cent to 8 per cent of all the cases.[14, 15]

The fetus and the newborn infant.

Factors favoring fetal infection. Bacteria proliferate in the amniotic fluid, or, after rupture of the membranes, on the amniotic and fetal surfaces. The skin offers a good protection against bacterial penetration. Bacteria are swallowed (Figs. 14, 15) or aspirated in the respiratory tract. Even without swallowing or respiratory movements, bacterial invasion may probably take place along the continuous column or film of fluid extending from the amniotic cavity to the fetal tissues.

It has not been demonstrated that intrauterine asphyxia increases the incidence or severity of fetal infection although it appears sensible to think of intrauterine gasping as a factor in more massive inoculation. Particulate amniotic contents (vernix, other epithelial cells, and meconium) are found very frequently in considerable amounts in association with fetal or neonatal pneumonia,[53, 60, 73] but this is not a consistent

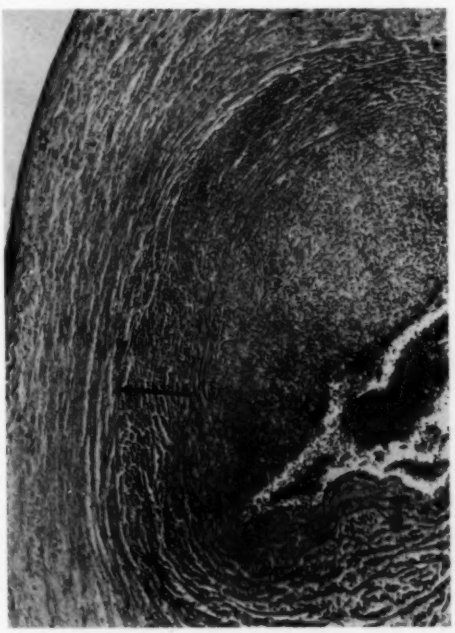


Fig. 13. Severe arteritis of the umbilical cord with parietal thrombosis. The neutrophils migrate in the direction of the arrow toward the amnion. (Hematoxylin and eosin. ×100; reduced ⅓.)

Table III. Association of pneumonia and placentitis in 253 autopsies of stillborn and 1-day-old infants (modified from[15])

Degree of placental inflammation*		*Aspiration of amnitic exudate*		*Pneumonia*		*% of pneumonia*
Macerated stillborn infants						
None	46	0		0		
Slight	28	2		1		
Marked	2	1		0		
	76		3		1	1
Nonmacerated stillborn infants						
None	65	0		0		
Slight	13	5		2		
Marked	40	15		4		
	118		20		6	5
Liveborn infants, 0-24 hours						
None	44	1		1		
Slight	2	0		0		
Marked	13	11		7		
	59		12		8	14
Total						
None	155	1		1		
Slight	43	7		3		
Marked	55	27		11		
	253		35		15	6

*Slight = membranitis and intervillositis; marked = chorioamnionitis.

feature.[60] Ahvenainen[72] found no difference in the incidences of neonatal pneumonia after normal and complicated labor, but the causes of complication more likely to result in infection were not analyzed separately. Inhalation of particulate amniotic material could be irritating and produce chemical inflammation.[74] This may be due to the bile content in meconium.[47]* There is, however, no clear-cut evidence that sterile amniotic fluid produces a neutrophilic response.[15, 47, 68, 75]

The passage through the birth canal would appear in itself to increase the danger of infection by aspiration of vaginal bacteria. It is not known, however, if contamination of the oropharynx actually takes place in the vagina. This source of infection was denied long ago.[3] More recently, no significant difference in the incidence of pneumonia could be demonstrated between babies delivered spontaneously and by elective cesarean section.[72]

*Page 69.

Fetal sepsis is probably associated with massive contamination of the gastrointestinal or respiratory tracts[18, 20, 76, 77] and not with simultaneous maternal sepsis and transplacental spread, since maternal bacteremia, frequent after delivery, is very rare during labor.[20]

There is progressive gradation from the "physiologic" colonization or contamination of the newborn during or immediately before labor to severe amniotic infection with septic fetal or neonatal lesions. It is not known if these differences are due to individual variations in cellular or humoral resistance[47] or to factors such as the size of the inoculum or the virulence of the bacteria.

Bacteriology. The microorganisms found in the fetal or neonatal blood or within the lungs are those described above in amniotic infection and comprise again a number of "nonpathogens." In recent studies *E. coli*, Paracolobactrum, *Aerobacter aerogenes,* nonhemolytic streptococcus, coagulase-negative *S. aureus* and *S. albus* have been found.[58, 61]

The finding of bacteria such as *E. coli* is strongly suggestive of an infection acquired pre- or intranatally, but this is not a sufficient criterion since postnatal infections by the same organisms are also common.[47, 58] The same reasoning applies to the other bacteria of the birth canal which are also frequently found in the amniotic fluid or in the neonatal organs. It is also conceivable, but difficult to demonstrate, that a prenatal infection might become of clinical importance only at the age of a few days, particularly if antibiotics have been administered for some time after birth.

PATHOLOGY. A variety of findings are associated with amniotic infection, and bacteria, amniotic exudate or fetal lesions are found in the paranasal sinuses, the middle ears, the respiratory and gastrointestinal tracts. Any focal lesion might result in septicemia.

Congenital otitis and sinusitis. These have been well studied by Benner[78] and Hemsath[79] and show a striking association with pneumonia and with placentitis.

Congenital meningitis. Leptomeningitis may be present at or shortly after birth. It results either from hematogenous spread or follows otitis.[79-82]

Congenital ophthalmia and dermatitis. These are very rare complications of amniotic infection.[18, 63]

Congenital gastroenteritis. Acute gastritis, focal ileitis, and even fetal or early neonatal peritonitis have been described.[12, 13, 15, 77]

Congenital sepsis. Bacteremia is common in cases of amniotic infection.[20] Focal septic lesions are not frequent in autopsy series,[18] except in fetuses smaller than 30 cm. crown-heel.[18, 76, 77]

Congenital and neonatal pneumonia. Pneumonia is the most important complication of amniotic infection in the fetus and in the newborn,[3, 15, 18, 58, 60, 61, 72, 83] and is said to account for about 90 per cent of the fatal neonatal infections.[84] Contamination of the lung occurs in utero in fetal and in early neonatal pneumonia and might occur in utero, in the vagina, or postnatally in neonatal pneumonia. It is impossible to determine exactly the respective role of amniotic infection and of infection acquired after birth in the pathogenesis of neonatal pneumonia. About 50 per cent of neonatal pneumonia is considered to be acquired in utero.[84] Cases which appear within 24 hours after birth are almost certainly congenital[15, 72] and possibly all those found in autopsies up to 3 days of age.[47, 78, 80, 85, 86] Acquired pneumonia may, however, develop in a newborn in less than 24 hours.[72] In recent studies,[85, 87, 88] one peak of incidence is noted in the first 48 hours and a second one at a later time. This observation provides some circumstantial evidence for a link with antenatal infection. Other factors may be involved, particularly in premature infants, such as, central respiratory failure associated with brain damage, "respiratory distress," or massive aspiration of amniotic fluid, each of which might enhance infection.[59]

Fig. 14. Stomach of a newborn infant whose death was due to the amniotic infection syndrome. The gastric lumen is at the top and contains a plug of amniotic exudate made up of polymorphonuclear leukocytes, squames, and myriads of bacteria. (Hematoxylin and eosin. ×240; reduced ⅙.)

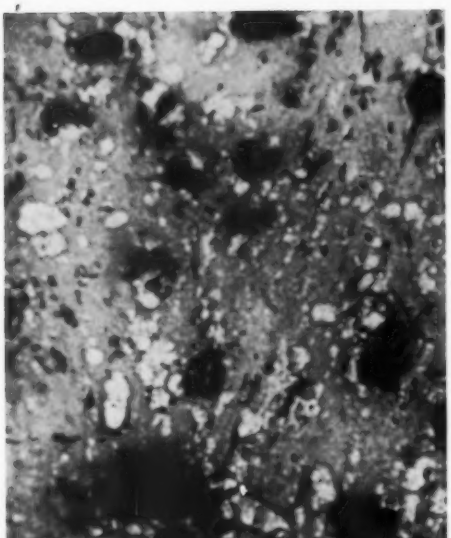


Fig. 15. Smear of gastric aspiration in a normal newborn infant, whose placenta showed chorioamnionitis. Note the segmented neutrophils and the bacteria. There is one epithelial cell at the left lower corner. The infant received no treatment and showed no sign of infection. (Methylene blue. ×900.)

Bacteriologic studies do not help to solve the problem of the correlation of neonatal pneumonia with amniotic infection for reasons mentioned above. For some authors, the bacteria found in the nasopharynx of newborn infants are similar to those found in their mothers' vaginas[89]; others believe that these bacteria are acquired by the aerogenous route.[90, 91] The two most recent studies[58, 61] reach opposite conclusions although the predominant bacteria are the same in both series. In one,[61] almost all cases are thought to be of pre- or intranatal origin, whereas, in the other[58] it is thought that no information as to origin of infection can be derived from their data. The growing importance of "nonpathogens" is illustrated in recent articles.[92-95]

The association of pneumonia with placentitis provides more circumstantial evidence of the link between pulmonary lesions and prenatal infection. This correlation was well established long ago and repeatedly confirmed. Johnson and Meyer found chorioamnionitis in 11 of 13 placentas examined in 30 cases of pneumonia in stillborn infants and in 6 of 9 placentas of infants with pneumonia who died within the first 4 days of life. All of the cases of pneumonia in Sorba's series in which the placenta was examined were found to be associated with funisitis and chorioamnionitis. In another series (Table III), the correlation of pneumonia and placental inflammation was highly significant.[15]

In a prospective study of 1,000 deliveries, the instances of pneumonia discovered at autopsy in infants up to 3 days of age were associated with placentitis, whereas after 3 days of age they were not.[15]

Another prospective study in 1,000 consecutive deliveries[15*] showed that of 9 neonatal deaths occurring in the first 48 hours, 3 were due to pneumonia associated with amniotic infection. It appears from Table III, that, at least within the first 24 hours of life, amniotic infection is a prerequisite to the aspiration of infected amniotic fluid, which, in turn, will produce pneumonia in some instances.

Morrison found placentitis in 88 per cent of his cases of pneumonia in stillborn infants and in 66 per cent of his series of neonatal pneumonia in infants up to 3 days of age. It seems that congenital pneumonia, or pneumonia of the newborn infant within the first 2 days of life, usually occurs in association with chorioamnionitis and funisitis. The absence of placentitis does not necessarily imply that infection occurred after birth, since it is conceivable that fulminating sepsis or pneumonia may develop in a fetus before amniotic inflammation. This situation was present only once in 15 instances of pneumonia in infants dying on the first day of life (Table III).

Benirschke has found neonatal infection to be five times as common in cases of pla-

*Page 730.

centitis as in cases with a normal placenta. Similarly, both morbidity and mortality rates in the infected group were twice as high as in the noninfected group.[16]

All these findings strengthen the notion that placental and cord examinations would provide a good means of evaluating the risk incurred by a given infant.

PATHOLOGY OF PNEUMONIA. In most instances, pneumonia is easily differentiated from aspiration of amniotic exudate by the presence of active leukopedesis.[18]

Recent descriptions[53, 60, 61, 83] have added little to classic papers.[1, 3, 4, 18, 96] The majority of the cases of congenital or early neonatal pneumonia are diffuse and acute with focal bronchiolitis and interstitial infiltrates (Figs. 16, 17, 18). Occasional cases, usually in small premature infants, show severe peribronchial lesions.[70, 76] The hematogenous type shows predominantly interstitial lesions. A necrotizing vasculitis has been found associated with Pseudomonas infection.[61]

In fetuses of less than 25 cm. crown-heel length, the inflammatory reaction is essentially monocytic or myelocytic.

INCIDENCE AND PRACTICAL IMPORTANCE OF PNEUMONIA IN THE STILLBORN AND NEWBORN INFANT. It appears difficult to conceive of pneumonia as a cause of death[15] of the stillborn infant, although this is maintained to be the case by some authors.[97] The incidence of pneumonia in the nonmacerated stillborn infants varied between 5 and 10 per cent.[15, 16, 18]

Pneumonia is considered by some to be responsible for 10 per cent of perinatal deaths.[58, 88] Others seem to think that pneumonia is somewhat less common as the main cause of death. Nesbitt[86] attributes 3 per cent of neonatal deaths to this condition. In two different studies[15, 98] it was found at autopsy that pneumonia associated with intrauterine infection was the cause of death in 5 per cent of the infants who died within the first 24 hours of life; in another 5 per cent, pneumonia was a contributing factor. Ten to 15 per cent of the total neonatal mortality is attributed to pneumonia.[72, 83]

In summary, amniotic infection by bacteria of the birth canal occurs frequently and produces a chorioamnionitis in up to 9 per cent of all deliveries. The majority of perinatal infectious lesions, and particularly pneumonia observed within the first 48 hours of life, occurred in cases of amniotic infection and were probably acquired in utero. These cases represent 5 to 10 per cent of all perinatal deaths. All infants exposed to amniotic infection have had contact with bacteria, but only a few develop intrauterine or neonatal infectious lesions.

The ratio of contamination as opposed to fatal congenital pneumonia or early neonatal pneumonia is estimated to be between 30 to 1 and 100 to 1. The fetuses are exposed to different bacteria or combinations of bacteria, and both the severity of the contamination and the duration of exposure vary. The role of these three factors and of the

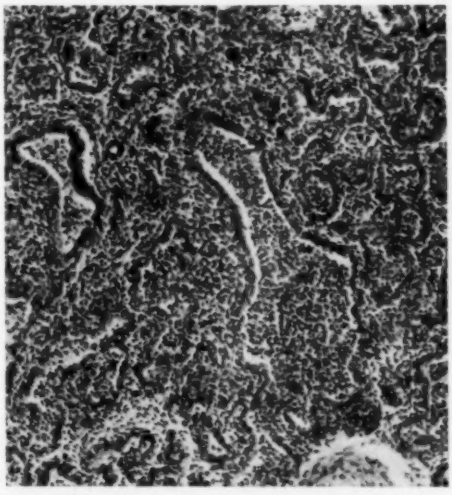


Fig. 16. Massive aspiration of amniotic exudate with early pneumonia. An infant 2 hours of age, weighing 540 grams, with amniotic infection syndrome. The membranes ruptured 12 hours ante partum; labor lasted 3 hours, 20 minutes. Plugs of markedly segmented degenerating neutrophils fill the air spaces. Only an occasional fetal neutrophil is seen in the pulmonary mesenchyma. In such a case, death is probably due to prematurity and massive aspiration of amniotic exudate and not to pneumonia. (Hematoxylin and eosin. ×200; reduced ⅓.)

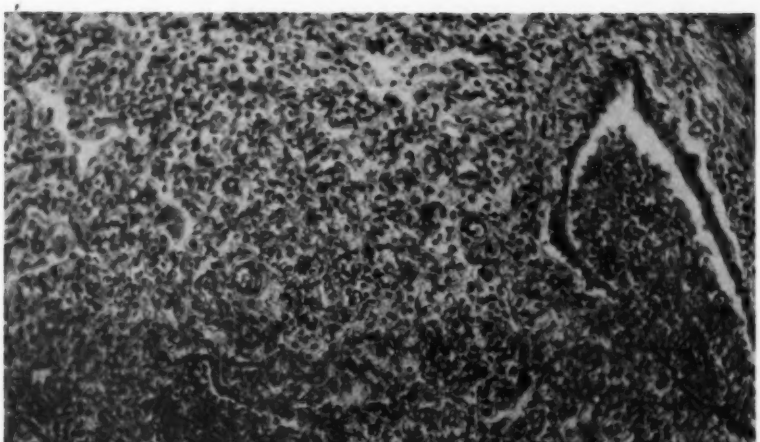


Fig. 17. Massive aspiration of vernix caseosa and amniotic exudate with diffuse early fetal reaction (congenital pneumonia) in a stillborn infant weighing 1,100 grams. Duration of labor: 7 hours without premature rupture of the membranes. There was a severe amniotic infection syndrome. Note the characteristic plugs of degenerating polymorphonuclear cells and amorphous debris in the bronchioles, the numerous pale squames in the alveoli, and the polymorphonuclear cells in the alveolar septi. Death was attributed to intrauterine asphyxia. (Hematoxylin and eosin. ×200.)

individual resistance of the fetus and of the newborn infant have not yet been evaluated.

AMNIOTIC INFECTION DUE TO CANDIDA ALBICANS. Intrapartum contamination of the newborn by *C. albicans* is common and probably occurs during delivery through an infected vagina.[99, 100] Infection before this time is very rarely recognized. It is secondary to an ascending infection of the amniotic cavity since transplacental moniliasis does not occur.[19]

Burry[101] published a case of hydrocephalus in a 37-day-old infant associated with the presence in ependymal granulation tissue of PAS (periodic acid–Schiff stain) positive hyphae and spores. It was not proved that infection took place prenatally in this patient.

To date, only one case of congenital cutaneous candidiasis has been observed.[102] Moniliasis of fetal adnexae was described 3 times (cord and placenta,[103] placenta,[105] cord[106]). There was no premature rupture of the fetal membranes in any of these 3 cases so that infection is supposed to have developed after subclinical rupture. Cultures were made in one instance[103] and *C. albicans* was the only organism grown from scrapings of the cord and placenta; hyphae were identified microscopically in the cord only. Histologically, the picture was that of a severe chorioamnionitis and funisitis.

No case of complete amniotic infection syndrome associated with *C. albicans* has been published. Such a case has been examined recently in Sloane Hospital, and showed funisitis and chorioamnionitis due to Candida and enteric bacilli.

The mother was admitted repeatedly for vaginal bleeding and had vaginal moniliasis. The breech delivery was rapid; the membranes had not ruptured prematurely. The full-term infant died 3 hours after birth in severe respiratory distress. The diagnosis at autopsy was severe congenital pneumonia due to enteric bacilli and *C. albicans*. The fungus was grown from the lung, amnion, and chorion, and identified histologically in the amnion, chorion, umbilical cord, lungs, stomach, and even in the lower ileum. On the amnion, the inflammation had a rather characteristic multifocal distribution corresponding to areas of massive fungal growth (Figs. 19-22).

It is likely that a number of cases of neonatal moniliasis and of pneumonia associated with the presence of *C. albicans*[72, 106] may be traced to intrapartum infection.

Neonatal mucormycosis[107, 108] and aspergillosis[109] appear unrelated to intrauterine contamination.

DIAGNOSIS OF AMNIOTIC INFECTION

In the mother. The diagnosis is suspected in the presence of any of the factors favoring ascending infection and of maternal pyrexia. Smith and others[57] considered infection likely if the maternal temperature is above 98.6° F. The correlation of the clinical suspicion and of placentitis is not constant, however.[12, 15]

In the fetus. It has been suggested, also by Smith and others, that persistent fetal tachycardia above 160 per minute was indicative of amniotic infection. In a preliminary study[15] neonatal leukocytosis did not appear to correlate with placentitis.

In the newborn. The diagnosis of infection related to amniotic infection is sus-

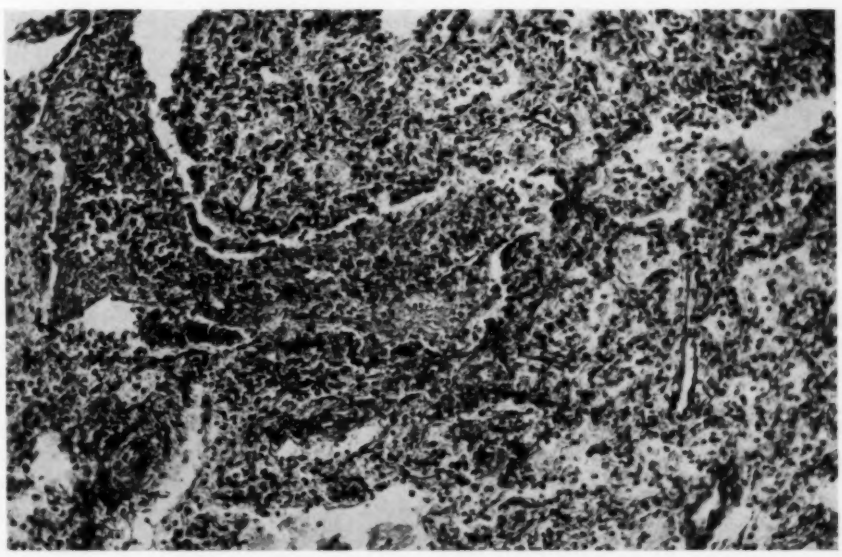


Fig. 18. Aspiration of amniotic material and congenital pneumonia in a 3-hour-old, 3,850 gram infant. Paracolon and *E. intermedia bacteria* were grown from the lung. Severe amniotic infection syndrome in an infant born after a labor of only 1 hour, 13 minutes, and rupture of the membranes at full dilatation. Respiratory distress from the time of birth. Death attributed to congenital pneumonia. (Hematoxylin and eosin. ×240; reduced ¼.)

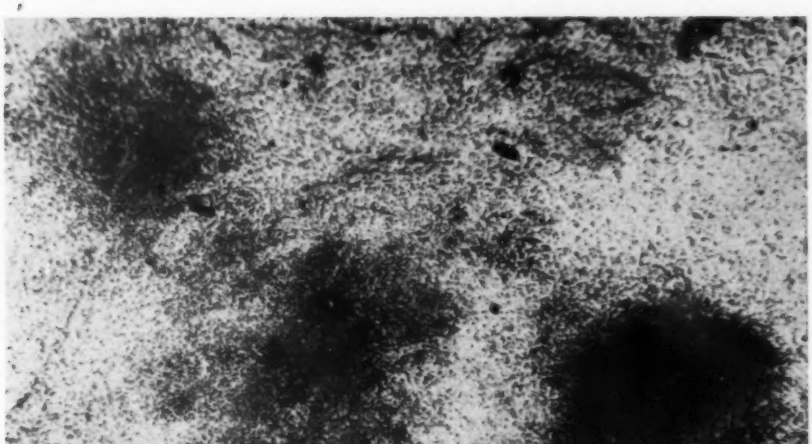


Fig. 19. Amniotic infection due to enteric bacilli and *Candida albicans*. Whole mount of amnion showing four foci of massive inflammation (all found centered with colonies of Candida). (Cresyl violet, ×100.)

pected after prolonged labor with ruptured membranes, prolonged labor,[110] and severe maternal pyrexia. The clinical diagnosis of sepsis or pneumonia in the newborn infant is difficult[10, 58, 72, 83, 85] and signs and symptoms are frequently indefinite.[10] The best policy appears to be: have a high index of suspicion, observe closely, and make a thorough search for bacteria.[62, 111]

Morphologic tests of diagnosis. The association of chorioamnionitis with fetal neonatal infection has led to the use of placental or cord examination as a means of determining exposure of the infant to infection. The finding of inflammation of the fetal adnexa is no indication of fetal disease, but points to the likelihood of bacterial contamination. As such, all morphologic tests of amniotic infection should be used in conjunction with other clinical evidence. It also seems logical to suggest that the systematic application of these tests to all newborn infants would draw the attention of the pediatrician to the group most likely to develop septic complications in the immediate neonatal period. At the present time, these techniques provide the only quick objective means of obtaining presumptive evidence of amniotic infection.

Chorionic imprint and gastric smear. Both the chorionic imprint and gastric smear tests[12, 13] have limitations and are now replaced by a frozen section of the umbilical cord or of a whole mount of the amnion.

Frozen section of the umbilical cord. A frozen section of the umbilical cord is a very satisfactory test, particularly if the quick method proposed by Benirschke and Clifford[14] is used. Funisitis is almost always asso-

ciated with chorionitis. The cord is easy to cut and good permanent sections can be obtained.

Whole mount of the amnion. The only advantage of the whole mount technique is that it does not require special equipment. It is based on the fact that neutrophils accumulate beneath the amnion in chorioamnionitis (Figs. 11, 23). Thus, a moderate fetal reaction at the earlier stage of vasculitis will not be diagnosed by this method.

A square inch of amnion is taken from the placental surface in an area overlying chorionic vessels. It is fixed in 95 per cent alcohol for one minute, floated in water, stained one minute in 1 per cent aqueous cresyl violet, rinsed in water, and mounted on a slide like a frozen section. In order to be sure that the undersurface of the amnion is facing up, one may check the slide microscopically or simply cut the fragment of amnion in two and flip one of the halves upside down. While held with a glass rod, the fragments are flooded with 3 successive rinses of absolute alcohol (20 seconds each), and 2 rinses of toluol, and mounted in balsam or permount.

COMMENTS ABOUT PROPHYLAXIS AND THERAPY

The scant available information related to the problems of therapy and prophylaxis is summarized by Silverman.[10]

In the mother. Information concerning the passage of effective amounts of drugs into the amniotic cavity and fetal blood is still meager. To date, no extensive controlled study is available which compares the outcome of children of treated and untreated mothers. Some published studies point to a possible decrease in the number of fetal and neonatal deaths. Von Friesen[112] gave small doses of sulfamerazine during labor to mothers who had fever, prolonged labor, and primary inertia, and found that the number of perinatal deaths decreased by 50 per cent. Penicillin and streptomycin did not appear to influence the rate of infection in other series.[12, 113, 114] On the other hand, Smith and others obtained spectacular, but as yet unconfirmed results with the use of streptomycin (1 Gm. twice a day) and oxytetracycline (0.5 Gm. every 6 hours) to all mothers with fevers of 98.6° F. or more or with sustained fetal tachycardia of 160 or more per minute. In this group, there was a remarkable drop not only in the incidence of fetal and neonatal pneumonia, but also in neonatal deaths throughout the first week of life.

In the infant. Bound and co-workers[115] gave prophylactic chemotherapy (penicillin and streptomycin) to premature infants, in-

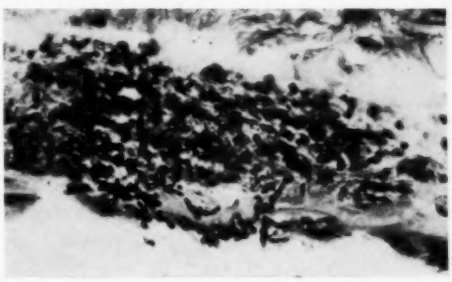


Fig. 20. Focal chorioamnionitis due to *Candida albicans*. The amniotic epithelium is largely destroyed and spores and hyphae are found within the chorion. (Periodic acid–Schiff. ×200; reduced ½.)

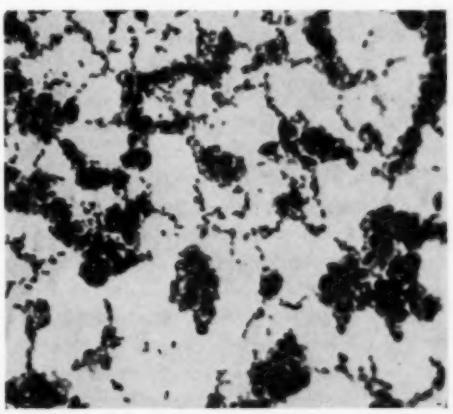


Fig. 21. Smear (postmortem) from gastric contents in amniotic infection due to *Candida albicans* and enteric bacilli. Note the large dark, gram-positive spores and the pale, gram-negative rods. (Gram, ×600.)

Fig. 22. Hyphae and spores of *Candida albicans* in the lumen of the lower ileum. (Periodic acid–Schiff. ×740.)

fants born after prolonged labor with or without fetal distress, or after prolonged rupture of the membranes (more than 6 hours), infants in poor condition at birth, and in cases of evidence of maternal infection. The incidence of pneumonia decreased from 16 to 6 per cent. A similar prophylactic regimen was found ineffective by others.[11,16]

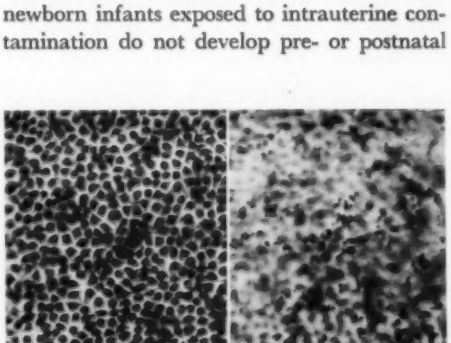
The present state of affairs with respect to premature infants is summarized by Silverman[10] as follows:

"It has not been established that the prophylactic administration of an anti-bacterial drug (or combination of drugs) to all premature infants who have been exposed to amnionitis is both safe and effective in reducing the number of deaths due to congenital bacterial infections. In the present state of our knowledge it can only be said that when a diagnosis of amniotic infection or umbilical vasculitis is made the infant should be closely observed for signs of infection (particularly respiratory difficulty) and treated with anti-bacterial drugs when the signs are present."

Some infants are born with respiratory difficulties produced by severe pneumonia and it is doubtful whether these babies can be saved by even the most energetic treatment. It is also clear that treatment should not be withheld in the presence of clear-cut infectious symptoms even if there is no evidence of amniotic infection. As soon as infection is suspected an attempt to identify the responsible bacteria should be undertaken in order to prescribe the most effective antibiotic.

SUMMARY AND CONCLUSIONS

Infection is still an important factor in perinatal death. Hematogenous transplacental infections may be due to a number of viruses, bacteria, or protozoa and produce a variety of fetal or neonatal lesions. Knowledge of pathologic changes in the placenta and of the natural history of these conditions is still far from complete. Transplacental infection does, however, play a limited role in perinatal death. Ascending placentofetal infection does not seem to be of much practical importance except in abortion and when combined with amniotic infection. The amniotic infection syndrome is a well-known pathologic and, to some extent, clinical entity observed usually, but not always, in infants born after prolonged labor and premature rupture of the membranes. It is due to bacteria of the birth canal and occurs in about 1 of 10 deliveries. Most fetuses and newborn infants exposed to intrauterine contamination do not develop pre- or postnatal



A *B*

Fig. 23. Whole mount of amnion in a case of chorioamnionitis. On the left *(A)* the picture is focused on the amniotic epithelium; on the right *(B)* it is focused on the upper chorion containing the polymorphonuclear leukocytes. (Cresyl violet. Original magnification ×340; reduced ⅓.)

infectious lesions. In such cases, the only evidence of amniotic infection is the finding of chorioamnionitis or funisitis. On the other hand, congenital infections and probably the majority of infections of the first 2 or 3 days of life develop in the group with chorioamnionitis. Congenital or neonatal infections, particularly pneumonia associated with amniotic infection, are found as primary or contributory causes of perinatal death in about 10 per cent of the infants examined at autopsy. No agreement has yet been reached as to the best means of prevention or treatment of such infections.[117]

The diagnosis of congenital and early neonatal infections is difficult, and the systematic prophylactic administration of antibiotics is potentially harmful. Its dangers have to be weighed against the risk of infection. Such a risk appears considerably greater in cases of infection of the amnion which should be observed closely.

Exposure to intrapartum infection is one of the points to be considered in a rational approach to prophylaxis and therapy of neonatal infection. Evidence of exposure to amniotic infection can now be obtained rapidly at birth by the use of simple and reliable tests based on the demonstration of inflammation in the umbilical cord or in the placenta. Rapid identification of the bacteria responsible for the infection should be attempted.

Ideally the tests should be performed routinely, or at least on all premature infants because of the higher incidence of amnionitis in these infants. It is suggested that prospective controlled studies of the value of prophylactic or therapeutic regimens be based on objective criteria of amniotic inflammation.

One of the puzzling aspects of the amniotic infection syndrome is the rarity of overt infection in infants who were certainly contaminated in utero. Whether the differences in the clinical course depend on differences in natural defense mechanism, degree of contamination, route of contamination, or type(s) of bacteria[118] is not known.

As illustrations of the routes of fetal infection and of the value of placental examination, unusual examples of transplacental and amniotic infection are described, i.e., a case of cytomegalic inclusion disease with viral placentitis, and one of an amniotic infection syndrome due to *Candida albicans* associated with enteric bacteria.

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An electron microscopic study of eccrine sweat glands in patients with cystic fibrosis of the pancreas

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CYSTIC fibrosis of the pancreas is a disease of unknown etiology and unknown pathogenesis despite intensive study in recent years. Andersen,[1] in the initial comprehensive description of the disease, thought the pancreatic lesion was primary and the pulmonary changes were secondary to dietary deficiency. The numerous causes postulated since her description have been recently reviewed.[2-4] The concept that this disease is due to an abnormal production of mucus and should be termed mucoviscidosis as put forth by Farber[5] is perhaps the most popular of current theories. The observations of Bodian[3] that the submaxillary and sublingual glands are also involved tended to support this proposed theory. Darling and associates[6] in 1953 described the functional involvement of sweat glands in cystic fibrosis, and this abnormality now provides the diagnostic test for the disease. Subsequent work has also demonstrated abnormal electrolyte concentrations in both lacrimal and parotid glands.[7-11] The finding of abnormal electrolyte concentrations in the secretions of sweat, lacrimal, and parotid glands has been used as an argument opposing the concept that a defect in mucus production is responsible for all the findings in this disease.[12]

Attempts to interpret the etiology and pathogenesis of this disease in a comprehensive manner have been hindered by the lack of understanding of the basic morphologic or biochemical abnormality. Histologic study of postmortem material has resulted in a compilation of data on the pathologic changes in various organs but has not explained the abnormal elaboration of electrolytes in glandular secretions. One approach to such a problem would be to examine the ultrastructure of one of the glands whose secretion is abnormal but evidences no morphologic change by light microscopy. The eccrine sweat glands are readily accessible for biopsy

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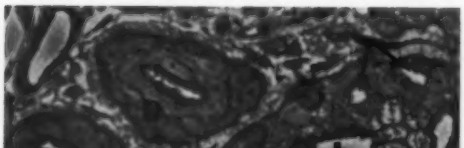

Fig. 1. Phase-contrast photomicrograph of eccrine sweat gland from a patient with cystic fibrosis. The secretory segment is cut tangentially, and 2 portions of the lumen *(L)* are pictured. Secretory vacuoles *(s)* are seen as round empty vesicles in the apex of certain cells termed mucoid cells. Clear cells are associated with intercellular canaliculi *(arrows)* and lack secretory vacuoles. Two portions of an eccrine duct *(D)* are present lined by a double layer of cuboidal cells. The cuticular border is the darker zone immediately surrounding the duct lumen. (×660.)

Fig. 2. Secretory segment of eccrine sweat gland stained with the colloidal iron stain for acid mucopolysaccharides and counterstained with nuclear fast red. Material in the photomicrograph which appears black is dark blue under the microscope, and the gray nuclei are red. The contents of the lumen *(L)* and the secretory vacuoles *(S)* are stained positively for mucopolysaccharide. (×1,150.)

from living patients,[13] and they comprise the basis of the present study to compare the morphology of normal human[15] and feline[14] eccrine sweat glands with those from patients with cystic fibrosis of the pancreas.

The ultrastructure of normal eccrine glands[14, 15] has already been described in both resting and secretory phases. To summarize these findings briefly: The human eccrine sweat glands are tubular glands extending from the skin surface to the dermosubcutaneous junction, where they are coiled into a loose mass. Two portions of the gland can be recognized, a secretory segment and a duct. The secretory segment is lined by 2 types of cells: mucoid (formerly termed dark[16]) cells, which secrete by means of secretory vacuoles a product containing an acid mucopolysaccharide, and clear cells, which secrete by means of intercellular canaliculi what is presumed to be a watery fluid. The duct is lined by a double layer of cuboidal cells termed surface cells (bordering the lumen) and basal cells (resting on the basement membrane). The luminal edge of surface cells is specialized into a cuticular border containing many tonofilaments. The basal duct cells abound with mitochondria, which contain many mitochondrial granules. The eccrine sweat gland of the cat differs in having a duct that is markedly shorter, and the duct cells contain only scant mitochondria; the secretory segment of the gland of the cat is identical with that of man. Since the cat secretes an isotonic sweat,[17] whereas human eccrine sweat is usually hypotonic, the human duct may be inferred to be responsible for the reabsorption of sodium and chloride from the precursor solution presented to the duct by the secretory segment.

MATERIALS AND METHODS

The patients in this study were from the hospital wards and outpatient clinic of the Harriet Lane Home of The Johns Hopkins Hospital. All subjects were proved cases of cystic fibrosis as evidenced by abnormal sweat electrolyte concentrations and radiologic pulmonary findings. One patient had fairly normal pancreatic function. Eight patients, ranging from 11 months to 8 years of age, were studied, 6 of these both before and after stimulation, and the other 2 in the resting state only. To stimulate secretion of

sweat the pilocarpine iontophoresis method of Gibson and Cooke[18] was employed, with 0.2 per cent pilocarpine nitrate used under the electrode and the current passed for 5 minutes. At 5, 15, and 30 minutes (2 cases each) after stimulation a cylinder of skin was removed without anesthesia, with the use of the high-speed rotary punch biopsy of Urbach and Shelley.[13] The volar aspect of the forearm was used as the donor site throughout.

Following removal of the biopsy the disc of skin was cut into smaller pieces and placed in Dalton's[19] chrome-osmium fixative for 1 hour at room temperature. The tissue was dehydrated through graded alcohols and embedded in either methacrylate or Epon 812 prepared according to Luft's[20] method. The methacrylate was polymerized overnight at 60° C. with benzoyl peroxide as the catalyst.

Sections were cut on a Porter-Blum micro-

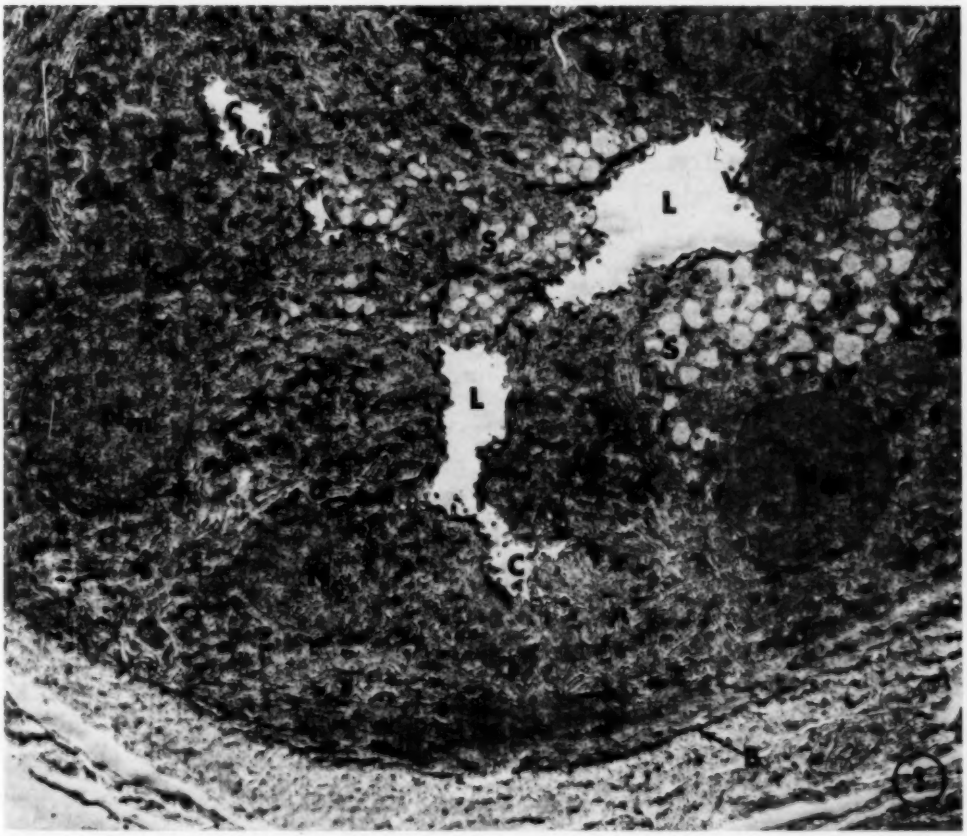

Fig. 3. Low-magnification electron micrograph of eccrine sweat gland secretory segment. The lumen *(L)* of the secretory segment is cut tangentially. Mucoid cell nuclei *(Nm)* and clear cell nuclei *(Nc)* can be identified on the basis of the cytoplasmic characteristics. Mucoid cells contain numerous apical secretory vacuoles *(S)*. Intracellular canaliculi *(C)* course between adjacent clear cells from the lumen *(L)* to within a micron or so from the base of the cells. Clear cells lack secretory vacuoles *(S)*. Lipid droplets *(F)* are present in both cell types, and both cells have small microvilli *(V)* projecting into the lumen or the intercellular canaliculi. Terminal bars *(T)* join adjacent cells as they border the lumen. Myoepithelial cells *(Y)* are present between the secretory cells and the basement membrane *(B)*. (×4,500.)

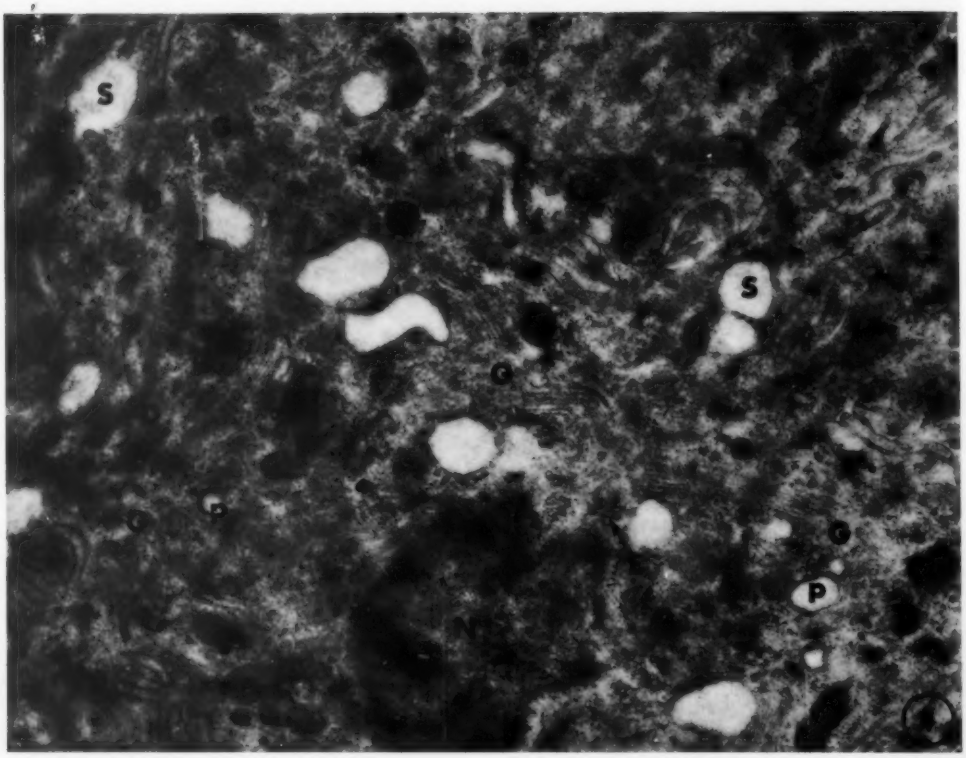

Fig. 4. Higher magnification of the cytoplasm and nucleus *(N)* of a mucoid cell. Secretory vacuoles *(S)* bounded by a definite limiting membrane and mitochondria *(M)* are scattered throughout the cytoplasm. An extensive Golgi apparatus *(G)* surrounds the nucleus *(N)*. Associated with the Golgi apparatus are small secretory vacuoles thought to represent secretory vacuoles in the process of formation; these are termed prosecretory vacuoles *(P)*. Desmosomes *(D)* connect adjacent cells to one another. Ergastoplasmic granules with a few associated membranous sacs *(E)* are scattered throughout the cytoplasm. (×22,500.)

tome with glass knives. Sections, 1 to 2 μ thick, of methacrylate-embedded tissue were mounted on glass slides for phase and light microscopy. Stains for light microscopy included the gallocyanin chrome alum stain for basophilia, a modified Hale colloidal iron stain for acid mucopolysaccharides, and the periodic acid-Schiff method for glycogen.[15] Thin sections of either methacrylate- or Epon 812-embedded tissue were mounted on formvar-coated grids and examined in either an RCA EMU-3B or -3D electron microscope. Selected sections of Epon-embedded tissue were stained with uranyl acetate[21] to increase electron contrast.

OBSERVATIONS

All observations and illustrations are from patients with cystic fibrosis of the pancreas unless specifically stated otherwise.

Light microscopy. The eccrine sweat glands from patients with cystic fibrosis appear normal by routine light or phase microscopy—a secretory segment bounded by a layer of columnar secretory cells and a duct bounded by a double layer of cuboidal cells (Fig. 1). Two cell types can be identified in the secretory segment, those with apical secretory vacuoles, termed mucoid cells,[15] and those without secretory vacuoles, termed clear cells. In sections stained with

gallocyanin chrome alum to demonstrate cytoplasmic and nuclear basophilia the cytoplasm of the mucoid cells is stained whereas that of the clear cells is unstained.[16] The intensity of cytoplasmic staining in such preparations appears to be somewhat less than normal. The secretory vacuoles of the mucoid cells stain positively for acid mucopolysaccharide when the Hale colloidal iron method[22] is used (Fig. 2). Fewer secretory vacuoles are consistently seen as compared to the normal human or feline eccrine glands,[14, 15] but such a change is very difficult to quantitate.

Electron microscopy of secretory segment. Two different types of cells can be identified in the secretory segment by low-magnification electron microscopy (Fig. 3). Cells with apical secretory vacuoles are the mucoid cells, and those cells lacking secretory vacuoles associated with intercellular canaliculi are the clear cells. Scattered throughout the apical cytoplasm of mucoid cells are secretory vacuoles rimmed by a limiting membrane (Fig. 4) and containing faintly fibrillar material. As seen by light microscopy, the number of secretory vacuoles is less than in the normal human, but the contents of the vacuoles appear to be identical with those of the normal.

The cytoplasm of mucoid cells is granular, and many of the granules are impossible to

Fig. 5. An intercellular canaliculus *(C)* between 2 clear cells stained with uranyl acetate to increase contrast. The intercellular canaliculus is bounded by numerous microvilli *(V)* and contains granular material. The cell membranes of adjacent clear cells interdigitate with one another, forming lamellar arrays *(I)* around the canaliculus. At the point where the adjacent clear cell membranes abut on the canaliculus the cells are joined by terminal bars *(T)*. Mitochondria *(M)* and small granules, many of which are probably glycogen in this stained section, are scattered throughout the cytoplasm. A portion of the nucleus *(N)* of one of the clear cells is present to the right. (×24,500.)

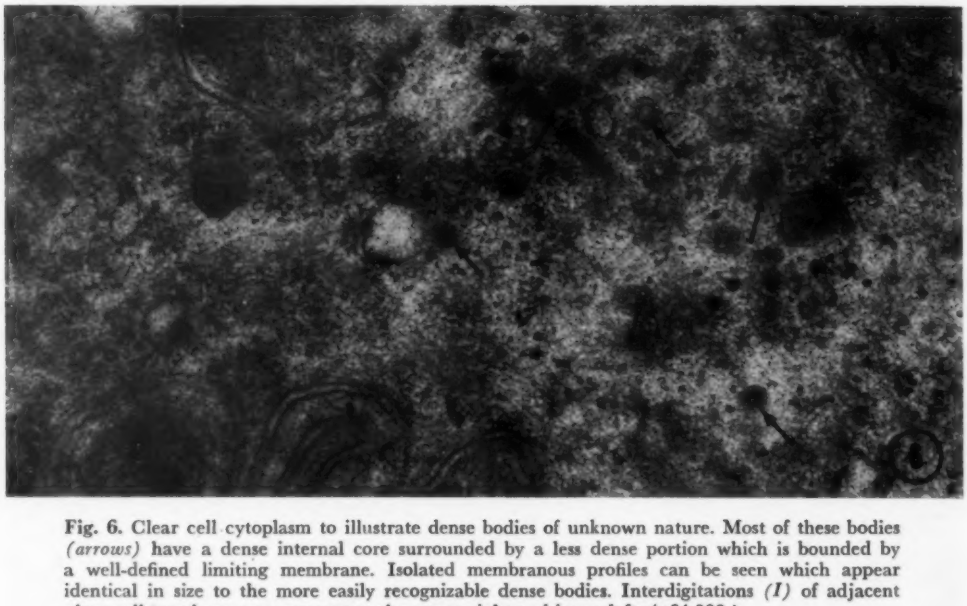

Fig. 6. Clear cell cytoplasm to illustrate dense bodies of unknown nature. Most of these bodies *(arrows)* have a dense internal core surrounded by a less dense portion which is bounded by a well-defined limiting membrane. Isolated membranous profiles can be seen which appear identical in size to the more easily recognizable dense bodies. Interdigitations *(I)* of adjacent clear cell membranes are present to the upper right and lower left. (×64,000.)

identify with certainty. Some of the granules appear identical with ergastoplasmic granules[23] thought to represent ribonucleic acid.[24] Occasional ergastoplasmic sacs with associated granules are present admixed with the free ergastoplasmic granules (Fig. 4). The Golgi apparatus is prominent in mucoid cells (Fig. 4) and consists of an array of flattened agranular membranous sacs, vesicles, and small vacuoles. In the midst of the Golgi apparatus are small vacuoles similar in appearance to secretory vacuoles, and these small vacuoles are considered to be prosecretory vacuoles (Fig. 4) and analogous to prozymogen granules[23] or immature beta granules[25] in pancreatic tissue. Mitochondria with rare mitochondrial granules are scattered throughout the cytoplasm. The apical cytoplasm bordering the lumen is thrown into irregular microvilli, and neighboring cells are joined at the luminal surface by terminal bars and elsewhere by scattered desmosomes (Fig. 3).

Clear cells are distinguished by the presence of intercellular canaliculi coursing between adjacent clear cells and the absence of secretory vacuoles in the cytoplasm (Fig. 3). Intercellular canaliculi are channels bounded by microvilli which extend from the lumen of the secretory segment to within a micron of the basement membrane of the gland (Fig. 3). Surrounding the canaliculi are multiple interdigitations of the adjacent cell membranes which form a lamellar system of membranes (Fig. 5). Adjacent clear cell membranes are joined by terminal bars as they abut on the canaliculus (Fig. 5), which proves the *inter*cellular nature of the canaliculi.

The cytoplasm of clear cells is granular, but the nature of the granules differs from those of mucoid cells in that they are for the most part larger, less dense, and less well defined, and only rarely are there ergastoplasmic granules (Fig. 5). Only scant membranous profiles of any type are seen in the cytoplasm of clear cells, and a Golgi apparatus is seen only infrequently (Fig. 7). Mitochondria are numerous and scattered throughout the cytoplasm.

The third cell type of the secretory segment is the myoepithelial cell, a smooth muscle cell located between the secretory cells and the basement membrane (Fig. 3). A detailed account of the structure of myoepithelial cells will be the subject of a future report.

Dense bodies 500 to 600 Å in diameter have been observed in all cells of the secretory segment (Fig. 6). These bodies have a central electron-dense core measuring around 500 Å and a delicate limiting membrane separated from the dense portion by a less dense space. Round membranous profiles lacking a central dense core, associated with the dense bodies, are also present. In the original description of human[15] and feline[14] eccrine glands these bodies were not noted, but upon re-examination of the original material identical dense bodies are present, although very rarely. These bodies in tissue from patients with cystic fibrosis are present in all cells—the mucoid, clear, and myoepithelial. No evidence has been found that these granules are associated with the secretory process following stimulation, and no association with age has been seen in the normal controls or patients with cystic fibrosis.

Electron microscopy of the eccrine duct. The wall of the eccrine duct is composed of

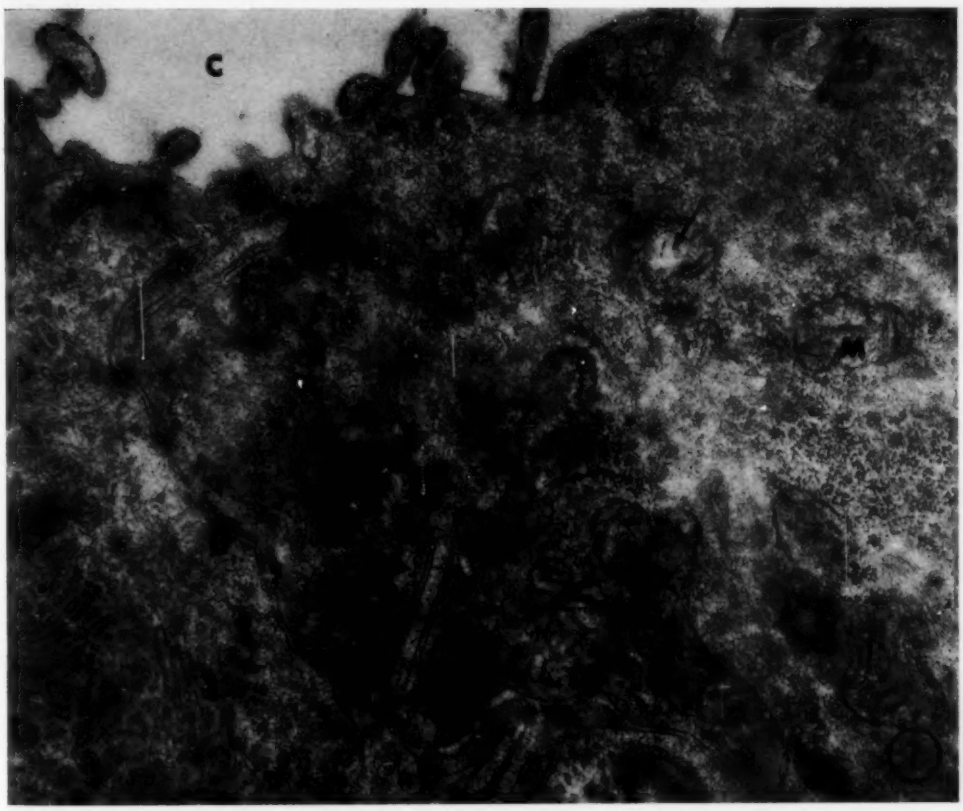

Fig. 7. Clear cell cytoplasm 30 minutes after stimulation. An intercellular canaliculus *(C)* bounded by microvilli *(V)* is present at the upper portion of the micrograph. The mitochondria *(M)* have areas of lessened density *(arrows)* in the matrix. A desmosome *(D)* is present between adjacent clear cells. A small Golgi apparatus *(G)* is present at the lower left in one clear cell. Many small granules of differing appearance are present in the cytoplasm. (×42,000.)

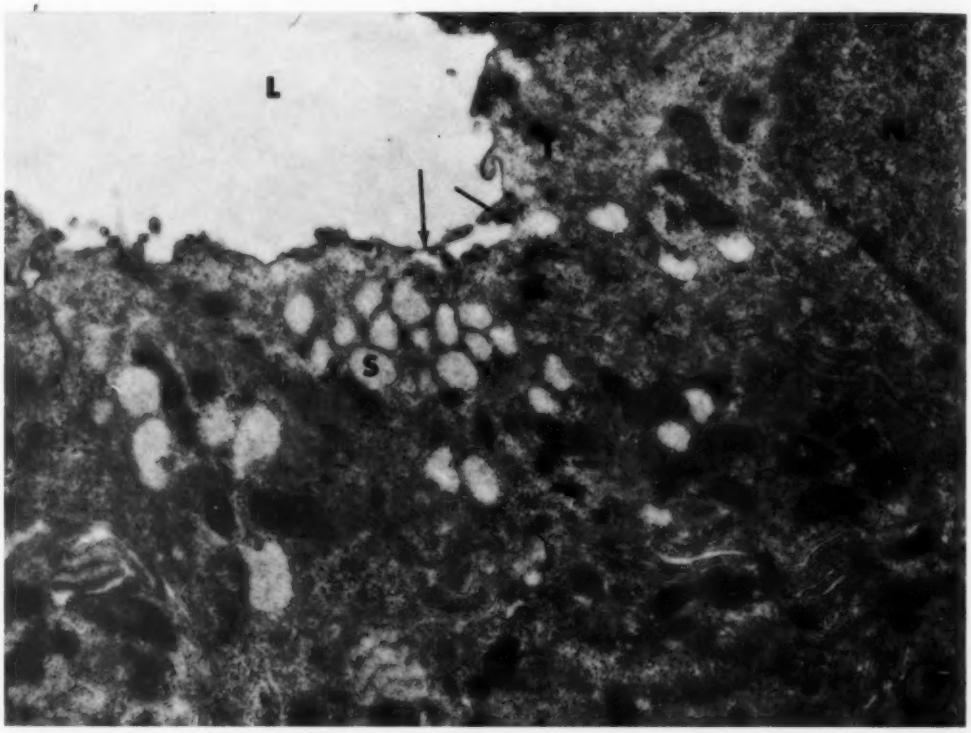

Fig. 8. Mucoid cells of the secretory segment 5 minutes after stimulation. The nucleus *(N)* of one mucoid cell is in the upper right. Numerous mitochondria *(M)* and secretory vacuoles *(S)* are present in the cytoplasm. Secretory vacuoles at the arrows appear to be in the process of being liberated from the cytoplasm into the lumen *(L)*. Terminal bars *(T)* join adjacent mucoid cells at the lumen. (×13,600.)

a double layer of cuboidal cells—one layer termed surface cells which line the lumen and the other termed basal cells that rest on the basement membrane (Fig. 9). Surface cells have a granular cytoplasm devoid of membranous elements. Mitochondria are scattered throughout the cytoplasm. The luminal edge of surface cells is specialized into what has been termed a cuticular border (Fig. 1). By electron microscopy the cuticular border is seen to be composed of filaments (Fig. 12) related to the numerous desmosomes which connect adjacent surface cells to one another and should be considered as tonofilaments. The structure of the desmosomes joining adjacent duct cells is identical to that described by Odland[26] for squamous epithelia (Fig. 10). The cell membrane of the surface cells bordering the lumen is thrown into small blunt microvilli (Fig. 12).

Basal cells in general appear similar in structure to surface cells. The cytoplasm is granular, and the cells interdigitate with one another and are joined by numerous desmosomes. However, mitochondria are much more abundant in basal cells, and many mitochondrial granules are present within these mitochondria (Fig. 11). The appearance of the cells of the duct and the mitochondria is identical with that of the normal gland. The basal cells rest on a distinct basement membrane (Figs. 9 and 11).

Electron microscopy of the eccrine gland following stimulation. Following stimulation by pilocarpine iontophoresis both cells of the secretory segment demonstrate alterations in morphology suggesting secretory activity. Mucoid cells evidence extrusion of secretory vacuoles into the lumen. The limiting membrane of the secretory vacuoles fuses with the cell membrane, and the contents of the vacuoles are liberated into the glandular lumen accompanied by bits of membranous debris (Fig. 8). These changes are identical with those in normal mucoid cells. Fewer secretory vacuoles are present following stimulation than in the normal cells, but again this change is very difficult to quantitate. No change in the dense bodies has been noted following stimulation.

Clear cells following stimulation show vacuolization of mitochondria as soon as 5 minutes after stimulation. This vacuolization appears to involve a lessening of the density of the mitochondrial matrix and leaves the cristae unchanged (Fig. 7). Occasionally a mass of glycogen rimmed by mitochondria is present, a finding seen so prominently in the cat following stimulation.[14] These changes in the clear cell are identical with those seen in the normal clear cell. No change in the dense bodies has been noted in clear cells following stimulation.

No changes have been identified in the

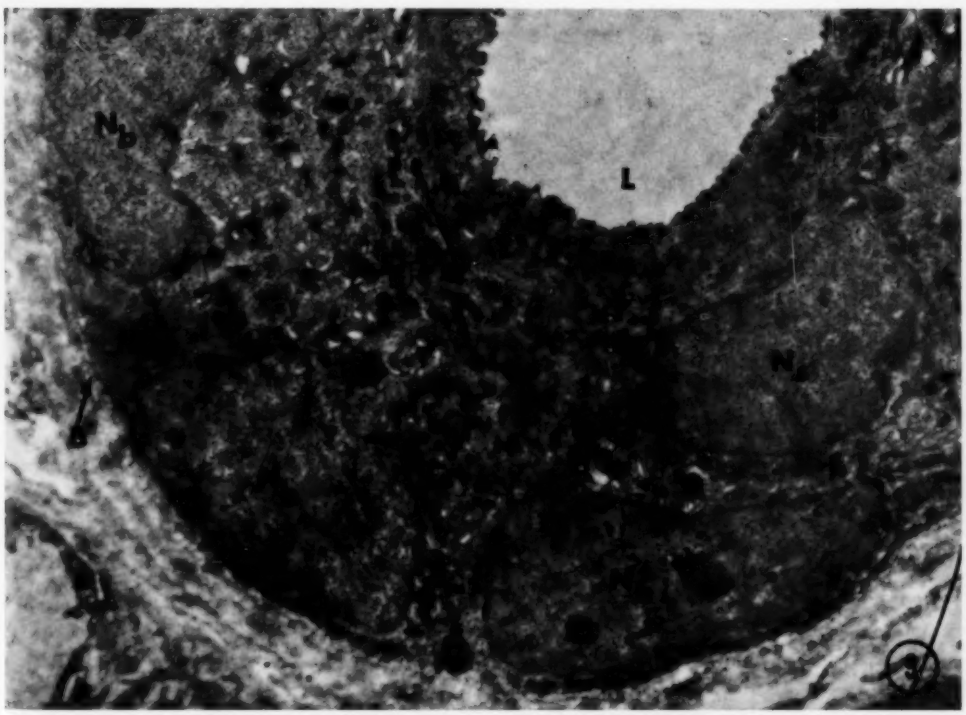

Fig. 9. Low-magnification electron micrograph of the eccrine duct. The dense cuticular border *(CB)* of surface cells surrounds the lumen *(L)*. The nuclei of one surface cell *(Ns)* and 3 basal cells *(Nb)* are present in this micrograph. Mitochondria *(M)* can scarcely be identified at this magnification, but structures identified positively as mitochondria at higher magnification can be seen in basal cells (see Fig. 10). Many desmosomes *(D)* join adjacent duct cells to one another. A basement membrane *(B)* surrounds the basal cells. A portion of an endothelial cell *(E)* of a capillary is present in the lower left. (×7,200.)

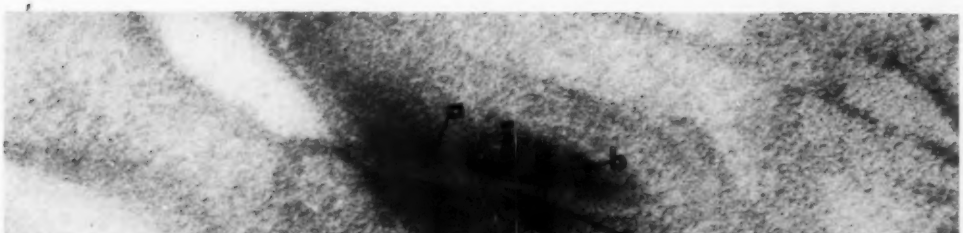

Fig. 10. Higher magnification of a desmosome from an eccrine duct. The apposed cell membranes *(a)* are thickened in the zone of the desmosome. Between the cell membranes *(a)* are three dense bands, the intercellular contact layer *(c)* and the intermediate dense layers *(b)*. On each side of the desmosome is a bundle of tonofilaments *(T)* inserting into the cell membranes. The cell membranes *(CM)* elsewhere in the micrograph are resolved into the unit membrane structure, that is, 2 dense lines separated by a clear space. (×132,000.)

eccrine duct following stimulation in either the normal or the present cases.

DISCUSSION

The current concept of the mode of eccrine sweat secretion delegates the elaboration of fluid to the two cell types of the secretory segment, mucoid cells and clear cells.[14, 15] This initial secretory product is most likely isotonic and becomes hypotonic by reabsorptive activity of the duct.[14] At high flow rates the tonicity increases, presumably due to a limited resorptive capacity of the duct. In cystic fibrosis, the failure of the eccrine glands to yield hypotonic sweat could theoretically be due to either of 2 different mechanisms: (1) deficient ductal reabsorption, or (2) elevated electrolyte concentration in the precursor fluid.

One of the cell types present in the secretory segment of eccrine sweat glands is an acid mucopolysaccharide-secreting cell termed the mucoid cell.[14, 15] The concept that mucoid cells secrete acid mucopolysaccharide rests on the observation that their cytoplasm is filled with secretory vacuoles which are liberated into the glandular lumen following stimulation and these vacuoles can be stained specifically[22] for acid mucopolysaccharide.[14, 15, 27] Additional evidence for a mucopolysaccharide secretion by eccrine sweat glands is shown by Jirka and Kotas,[28] who have demonstrated the presence of glucosamine in human eccrine sweat.

Chemical alterations in mucopolysaccharides from patients with cystic fibrosis have been described by Dische and coworkers[29] and Anderson and Freeman.[30] Since the mucopolysaccharides present in the eccrine sweat secretion could also be abnormal, 3 possibilities are offered as to how this abnormal mucopolysaccharide might affect the electrolyte concentration of sweat.

1. Mucopolysaccharides are polyelectrolytes and as such are capable of binding ions.[31-34] If the affinity is strong enough it is conceivable that the precursor fluid may either contain excessive electrolyte or bind the electrolyte in an unavailable state for distal reabsorption.

2. The hypothetical abnormal mucopolysaccharide may set up a mechanical barrier

to the reabsorptive site or injure the transport mechanism even to the extent that water might be permitted to diffuse passively into the duct with the electrolyte reabsorption.

3. Mucopolysaccharides possess an enormous hydrodynamic volume, in the case of large polymers exceeding 1,000,[31, 32] and this domain of water is not dependent on the osmotic properties of the molecule.[32] An alteration in the chemical structure of the mucopolysaccharide might result in less "bound" water, effecting a higher electrolyte concentration. Such a phenomenon should result in a decreased rate of sweating, but such has not been demonstrated.

None of these possibilities have taken into account the possible function of the epidermal portion of the sweat duct, which by recent evidence has been implicated in a possibly significant role in secretion.[35]

Other eccrine glands found to have abnormal electrolyte concentrations in cystic fibrosis of the pancreas (lacrimal and parotid)[11] also have mucopolysaccharide-secreting cells. Leblond[36] pointed out that rat lacrimal and salivary glands stain intensely with the periodic acid-Schiff technique for polysaccharide both before and *after* digestion with diastase and concluded that these acinar cells secrete a mucopolysaccharide. Such presumably serous cells secreting a polysaccharide Leblond[36] termed seromucoid cells. In both the cat and the human, the

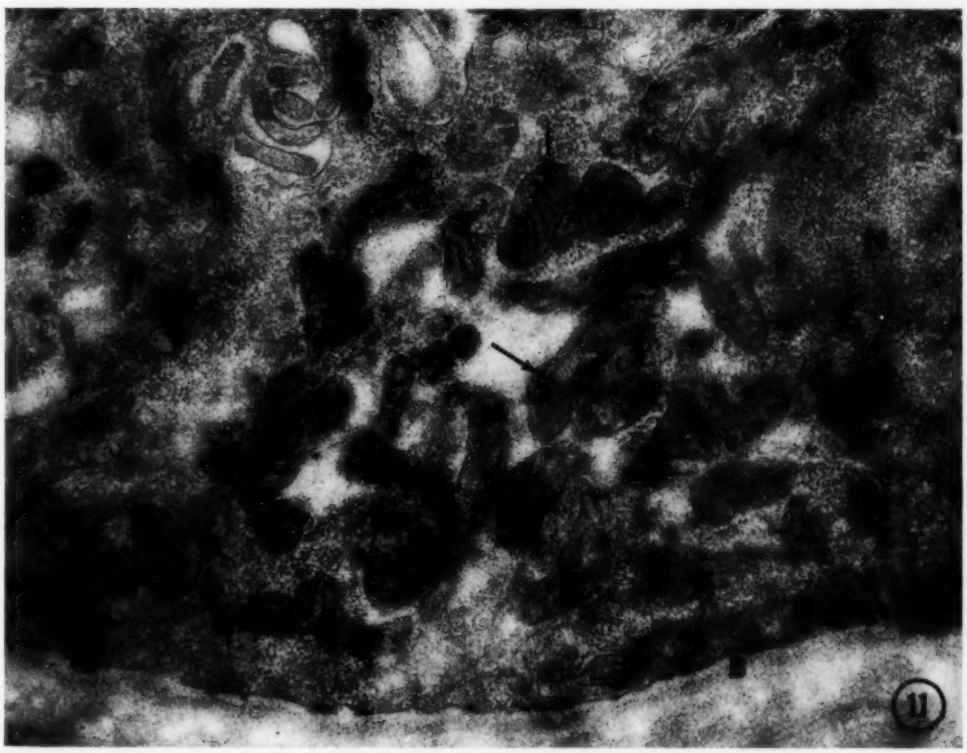

Fig. 11. Cytoplasm of basal eccrine duct cells. Numerous mitochondria *(M)* containing mitochondrial granules *(arrows)* are packed into the basal cell cytoplasm. The nucleus *(N)* of one basal cell is present to the right. Desmosomes *(D)* connect adjacent basal cells. A basement membrane *(B)* surrounds the basal cells. (×39,000.)

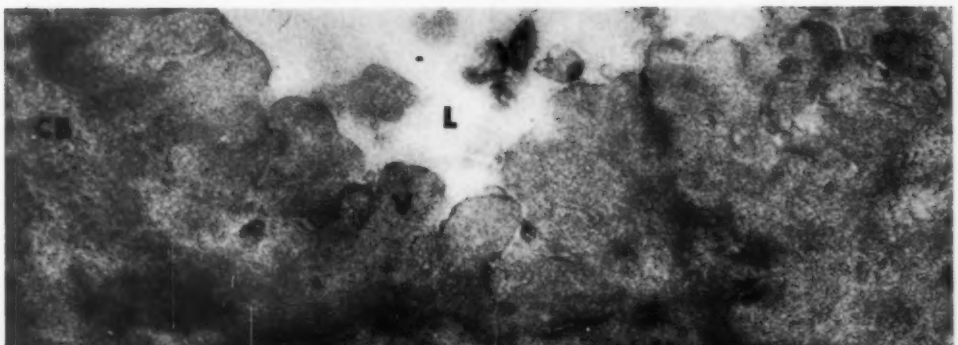

Fig. 12. The cuticular border of surface duct cells at higher magnification. The lumen *(L)* is bounded by blunt microvilli *(V)*. The cuticular border *(CB)* is a dense zone of compact filaments and granules. Note the tonofilaments *(arrow)* attaching to a desmosome *(D)* and the similarity between these tonofilaments and the filaments of the cuticular border elsewhere (compare with Fig. 10). (×42,000.)

lacrimal and parotid glands secrete a mucopolysaccharide,[37] and a detailed account of these findings will be the subject of another report. Thus the altered electrolyte of lacrimal and parotid glands in patients with cystic fibrosis of the pancreas can be explained on the basis of a mechanism identical with that of the eccrine sweat gland.

The pancreas is a true serous gland; it lacks demonstrable mucopolysaccharide in the acinar secretion,[36] but its ducts do contain numerous goblet cells. Dische and coworkers[29] postulated that the goblet cells of the pancreatic ducts could produce a thick mucus and the pancreatic changes present in cystic fibrosis of the pancreas would result from simple obstruction. All the changes of the pancreas in cystic fibrosis can be seen after pancreatic duct ligation.[38] The major and intrahepatic bile ducts also contain numerous goblet cells and a similar obstructive phenomenon due to thick mucus secretion would produce the changes occasionally seen in the liver in cystic fibrosis.[3]

Those organs which evidence morphologic alterations in cystic fibrosis of the pancreas contain mucus-secreting cells either in the secretory portion (submaxillary, sublingual,

Brunner's, and bronchial glands) or in the ductal system (pancreas and liver). A deficiency of pancreatic enzymes at birth, when these enzymes should be secreted,[23, 39, 40] results in meconium ileus, and later in life pancreatic deficiency is evidenced by morphologic changes in organs due to the lack of absorption of fat-soluble vitamins.[41, 42]

One unsolved problem of the morphology of eccrine sweat glands in cystic fibrosis of the pancreas relates to the identity and function of the dense bodies. These structures are very prominent in the eccrine glands in cystic fibrosis, and at first glance bear a resemblance to virus particles as seen by electron microscopy.[43] A viral etiology was postulated by Brody[44] after describing intranuclear inclusions in pancreatic acinar cells affected by the disease. Intranuclear inclusions are difficult to identify with certainty by light microscopy, however, and during the process of atrophy[38] or during embryologic development[23] marked changes are present in both nuclei and cytoplasm which could be confused with a virus inclusion.

Several arguments can be given as to why these structures, termed dense bodies, are not the causative factor in this disease, even if they are virus particles. First, they are present in all 3 cells of the secretory segment and an abnormality confined to the secretion of mucopolysaccharide would seem most likely. Second, no evidence of intranuclear particles has been seen. Finally, they are present, though rarely in normal cells. Since these particles resemble secretory granules of endocrine cells,[25] they are most likely a form of secretion, though they cannot be implicated in the secretory cells. In addition the Mendelian hereditary nature of the disease[3, 4, 45] tends to eliminate a virus as a causative agent, although it should be kept in mind that mouse mammary carcinoma, a viral disease, appears to have some hereditary characteristics.[46]

SUMMARY

Eccrine sweat glands contain an acid mucopolysaccharide-secreting cell in the secretory segment, the mucoid cell. Mucoid cells in cystic fibrosis of the pancreas contain fewer secretory vacuoles than normal. Unidentified dense bodies are present in all cells of the secretory segment in large numbers in cystic fibrosis, but they are also present in small numbers in the normal ones. The eccrine duct is normal in cystic fibrosis.

The basic defect in cystic fibrosis of the pancreas is an abnormality in the production of mucopolysaccharide. Those organs secreting mucus evidence morphologic alteration: the mucus-secreting goblet cells obstruct the duct of the pancreas and liver; and the mucus-secreting acini of bronchial, Brunner's, submaxillary, and sublingual glands produce obstruction and dilatation of these glands. Glands evidencing no morphologic alteration but demonstrating altered electrolyte secretion are seromucoid glands; that is, the secretory cells liberate low-viscosity material and are ordinarily considered to be serous, but mucopolysaccharide can be demonstrated in the acinar secretory products; the eccrine sweat glands, parotid, and lacrimal glands fall in this class.

Various hypotheses are presented to account for the defective electrolyte secretion of the seromucoid glands on the basis of altered mucopolysaccharide secretion.

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The flora of the respiratory tract of patients with cystic fibrosis of the pancreas

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CHRONIC pulmonary infection leading to pulmonary insufficiency is the principal cause of death of patients with cystic fibrosis of the pancreas.[1-6] Bacterial pathogens have been found to play a dominant role in the pulmonary involvement. These patients usually harbor the same bacterial pathogens for relatively long periods of time even in the event of marked clinical improvement. Such persistence of bacterial pathogens in the respiratory tract is not generally found in patients suffering from chronic respiratory infections due to other underlying causes.

The present report is a study of the incidence of bacterial pathogens in the different parts of the respiratory tract of patients with cystic fibrosis of the pancreas. This group of patients will be designated as CF patients in the remainder of this presentation. The results of a similar study in patients who have a chronic respiratory disorder not associated with cystic fibrosis, hereafter referred to as non-CF, are included for comparison. The data will be presented in the following order:

1. The bacterial pathogens cultured from randomly collected nasopharyngeal swabs and bronchial secretions obtained from CF patients from January, 1950, through April, 1958, compared with isolates obtained from a limited number of non-CF patients who underwent bronchoscopic examination in the same period of time (Group I).

2. A comparison of the bacterial pathogens from simultaneous nasopharyngeal and bronchoscopic cultures of CF and of non-CF patients from May, 1958, through September, 1959 (Group II).

3. A comparison of the pathogens isolated from simultaneous cultures from nasal, pharyngeal, nasopharyngeal, and bronchial secretions of CF and of non-CF patients from October, 1959, through September, 1960 (Group III).

4. A comparison of the bacteriophage patterns of a limited number of strains of *Staphylococcus aureus* isolated from CF and from non-CF patients.

5. The results of antibiotic susceptibility tests of a limited number of recently isolated strains of *Staph. aureus* and *Pseudomonas aeruginosa* from CF patients.

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MATERIAL AND METHODS

The respiratory flora of 83 CF patients were studied. Eight of these patients had been followed for a long time, and, therefore, were studied 3 times; 9 of these patients were studied twice, and the remaining patients were each studied once. Thus, a total of 108 studies were made on 83 CF patients.

The diagnosis of cystic fibrosis was established on the basis of characteristic clinical and roentgenologic manifestations of the disease and absence of tryptic activity in duodenal secretions of all CF patients studied. The diagnosis was further confirmed by postmortem examination in 31 cases. All patients whose diagnosis of cystic fibrosis was established after January, 1955, and all patients currently under observation were found to have elevated chloride concentrations in the sweat.

A total of 117 non-CF patients with a chronic respiratory disorder of infectious and noninfectious nature was investigated relative to the flora of the respiratory tract. Among these were 3 patients who were studied repeatedly in 3 periods of investigation, 4 patients who were studied twice, and the remaining patients who were studied once. Therefore, a total of 127 studies were made on 117 patients. Sweat chloride concentrations were obtained on a large number of non-CF patients; all were well within the normal range. The diagnoses of the non-CF patients were as follows: chronic bronchitis, 22; pneumonitis with or without atelectasis, 39; bronchopneumonia, 2; bronchiectasis, 14; bronchial asthma, 12; congenital heart disease with or without atelectasis, 3; pulmonary tuberculosis, 16; laryngeal stridor, 7; mediastinal mass, 1; and foreign body in esophagus, 1.

The age distribution of CF and non-CF patients and the number of deaths which occurred during the periods of observation are presented in Table I.

Nasal and pharyngeal cultures were obtained with sterile cotton applicators. Nasopharyngeal specimens were collected with wire applicators inserted into the nasopharynx through one of the nostrils and left in place for 30 seconds. All swabs were immersed in sterile trypticase soy broth for transport to the laboratory. Bronchial secretions were obtained during bronchoscopy by means of sterile Lukens collection tubes. All the random cultures taken before April, 1958, were planted on 2 sheep's blood agar plates, one of which was incubated aerobically and the other anaerobically. The cultures performed after April, 1958, were each planted on 2 sheep's blood agar plates, 1 levinthal plate, 1 eosin-methylene blue plate, and 1 Sabouraud's plate. All strains of *Staph. aureus* were tested for coagulase production by the tube method. A limited number of these strains which were isolated after April, 1958, were bacteriophage typed in the laboratory of Dr. K. M. Schreck.[7] A limited number of the same strains were also tested by the tube-dilution method for their antibiograms against 12 antibiotics, namely, bacitracin, chloramphenicol, erythromycin, novobiocin, oleandomycin, tetracycline, penicillin, streptomycin, kanamycin, neomycin, ristocetin, and vancomycin. A few strains of *Ps. aeruginosa* were tested against tetracycline, oxytetracycline, streptomycin, neomycin, polymyxin, chloramphenicol, and kanamycin by the same method.

Table I. Age distribution and number of deaths of CF and non-CF patients

	Groups		
	I	*II*	*III*
CF patients			
No.	50	30	28
Age	3.5 years	5.1 years	4.4 years
Range	2 months to 15 years	2 months to 15 years	3 months to 16 years
No. of deaths	22	5	5
Non-CF patients			
No.	22	58	47
Age	4.8 years	4.1 years	4.2 years
Range	2½ months to 12 years	3 weeks to 12 years	2 months to 14 years
No. of deaths	2	2	1

Table II. Results of cultures performed on CF and non-CF patients

	Group I		*Group II*		*Group III*	
	CF	*Non-CF*	*CF*	*Non-CF*	*CF*	*Non-CF*
No. of patients	50	22	30	58	28	47
Nasopharyngeal						
No. of cultures	209	65	113	97	71	71
Positive for pathogens (%)	75.1	47.7	65.5	53.6	78.8	62.0
Bronchial						
No. of cultures	162	79	113	97	71	71
Positive for pathogens (%)	97.5	69.6	99.1	58.8	93.0	67.6
Nasal						
No. of cultures	--	--	--	--	71	71
Positive for pathogens (%)	--	--	--	--	45.0	56.3
Pharyngeal						
No. of cultures	--	--	--	--	71	71
Positive for pathogens (%)	--	--	--	--	93.0	80.3

RESULTS

In order to avoid undue emphasis on the frequency of the bacteria repeatedly cultured from a few patients, only 10 cultures from each of those patients on whom many cultures were made were included in the analysis. Therefore, the number of cultures included in the analysis varied from 1 to 10 per patient in both CF and non-CF patients.

The results of the bacteriologic cultures were analyzed according to the presence or absence of the following pathogens: coagulase-positive *Staph. aureus,* beta hemolytic streptococci, pneumococci, coliform bacilli (including *Escherichia coli, Aerobacter aerogenes,* and *Klebsiella pneumoniae), Hemophilus influenzae, Ps. aeroginosa,* Bacilli proteus, and *Candida albicans.* Coagulase-negative *Staph. aureus, Neisseria catarrhalis, Streptococcus viridans,* diphtheroids, and yeasts were considered as normal flora.

The number of cultures obtained from the various sites of CF and non-CF patients and the incidence of pathogens in each group are shown in Table II.

GROUP I (FIG. 1)

The types of pathogens isolated from the CF and non-CF patients differed significantly. The predominant pathogens in both

Table III. Comparison of simultaneously obtained cultures of nasopharynx and bronchus

	CF		*Non-CF*	
Results of culture	*No. of paired cultures*	*%*	*No. of paired cultures*	*%*
Nasopharyngeal and bronchial, pairs	113		97	
Same pathogen in nasopharyngeal and bronchial	36	31.8	20	20.6
Different pathogens in nasopharyngeal and bronchial	13	11.5	12	12.4
Pathogens in nasopharyngeal only	0	0.0	15	15.5
Pathogens in bronchial only	40	35.4	17	17.6
Same pathogens in both and additional pathogen in nasopharyngeal	5	4.4	3	3.1
Same pathogens in both and additional pathogen in bronchial	18	15.9	5	5.1
Nonsignificant organisms in nasopharyngeal and bronchial	1	0.9	25	25.7

nasopharyngeal and bronchial cultures of CF patients were *Staph. aureus, Ps. aeruginosa,* and coliform organisms. In non-CF patients there was a relatively high incidence of pneumococci and of coagulase-positive *Staph. aureus* in both nasopharyngeal and bronchial cultures. The gram-negative pathogens isolated from the non-CF patients were approximately evenly distributed among *H. influenzae, Ps. aeruginosa,* and coliform organisms. The incidence of *Staph. aureus* in the bronchial secretions and *Ps. aeruginosa* in the nasopharyngeal and bronchial secretions of CF patients was significantly higher than in those of non-CF patients. However, the incidence of *Staph. aureus* in the nasopharyngeal cultures of CF patients was not significantly different from that of non-CF patients. The incidence of pneumococci and *H. influenzae* was significantly higher in non-CF than in CF patients.

GROUP II (FIG. 2)

These data are similar to those presented in Fig. 1. The incidences of *Staph. aureus* in the bronchial secretions and of *Ps. aeruginosa* in the nasopharyngeal and bronchial secretions of CF patients are again higher than those of non-CF patients. The bacterial pathogens isolated from non-CF patients do not follow a definite pattern. A detailed comparison of simultaneously obtained nasopharyngeal and bronchial cultures in Group II is presented in Table III. The higher incidence of pathogens in bronchial than in nasopharyngeal cultures of CF patients is significant. This difference is not demonstrated in the bronchial and nasopharyngeal cultures of non-CF patients.

GROUP III (FIGS. 3 AND 4)

A total of 71 cultures were obtained from each of 4 sites of the respiratory tract of 28 CF and 47-non-CF patients. *Staph. aureus* and *Ps. aeruginosa* were found in all four sites of CF patients, but the lowest incidence of bacterial pathogens was in the nasal cultures, which also showed the highest incidence of nonpathogenic organisms. There were relatively large numbers of pneumococci in the throat swabs and of coliform organisms in the bronchial secretions.

In the non-CF patients, the predominant pathogenic bacteria in the throat cultures were pneumococci, which were relatively infrequent in the bronchial cultures. *Staph. aureus,* which was not isolated as frequently as the pneumococci from throat cultures, was found about as frequently in the bronchial, nasopharyngeal, and nasal cultures as in those of the throat. Gram-negative bacilli were isolated with essentially the same frequency from the bronchial cultures as from those of the throat but with less frequency than from the nasal and nasopharyngeal cultures.

Staphylococcus aureus was the predominant bacterial pathogen in the nasal cultures

Table IV. Phage patterns of *Staphylococcus aureus* isolated from CF and non-CF patients

	CF		*Non-CF*	
	Nasopharyngeal	*Bronchial*	*Nasopharyngeal*	*Bronchial*
No. of strains tested	81	99	33	38
80/81	28	32	8	12
Not typable	18	29	12	7
Other types	35	38	13	19

Table V. *Staphylococcus aureus* inhibited by 12 antibiotics in quantities of 15.6 mcg. per milliliter or less as determined by tube-dilution method

	Nasopharyngeal		*Bronchial*	
Antibiotic	*No. tested*	*Sensitive (%)*	*No. tested*	*Sensitive (%)*
Bacitracin	90	96	101	99
Erythromycin	106	64	100	49
Oleandomycin	103	75	97	62
Novobiocin	105	80	101	80
Chloramphenicol	109	50	101	43
Kanamycin	101	50	85	53
Neomycin	99	76	89	64
Tetracycline	102	30	101	24
Streptomycin	106	31	87	26
Penicillin	107	28	88	17
Ristocetin	71	91	62	100
Vancomycin	89	94	62	100

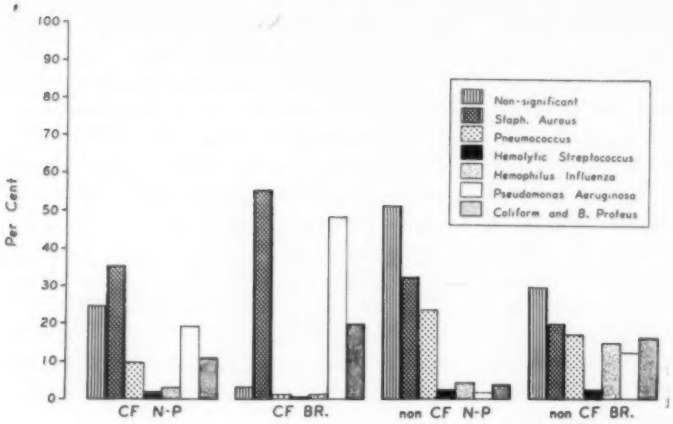

Fig. 1. Results of random nasopharyngeal and bronchial cultures of CF and non-CF patients.

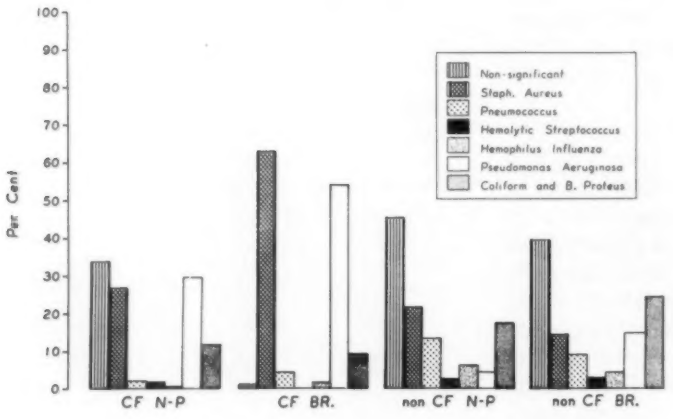

Fig. 2. Results of simultaneously obtained nasopharyngeal and bronchial cultures of CF and non-CF patients.

of non-CF patients. Although the incidence of nasal *Staph. aureus* in non-CF patients was higher than in CF patients, the difference is not statistically significant.

PHAGE PATTERNS OF STAPHYLOCOCCUS AUREUS

A limited number of strains of *Staph. aureus* isolated from nasopharyngeal and bronchial secretions of CF and non-CF patients were typed for their phage pattern. The results are summarized in Table IV. There were no significant differences in the phage types of *Staph. aureus* from CF and non-CF patients.

The phage patterns of *Staph. aureus* isolated simultaneously from different sources of the same individual were usually identical; however, in 6 of 25 instances of Group II CF patients, the phage types of the *Staph. aureus* isolated from the nasopharynx and from bronchial secretions were different. In 7 of 37 instances of Group III CF patients, the phage types of *Staph. aureus* present in the various sites of the respiratory tract were different as was also the case in 8 of 26 non-CF patients of Group III.

In individuals from whom *Staph. aureus* was isolated repeatedly, the same phage pattern was usually detected in CF patients; there was less consistency in the non-CF patients. The persistence of the same phage type in infected individuals will be presented in a later report.[8] The isolation of type 80/81

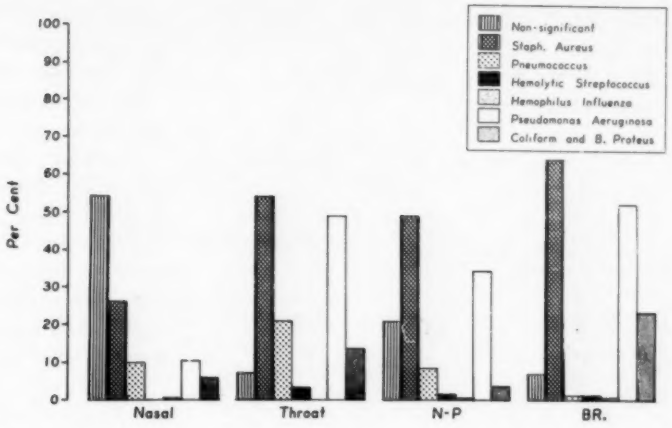

Fig. 3. Results of cultures obtained simultaneously from four sites of the respiratory tract of CF patients .

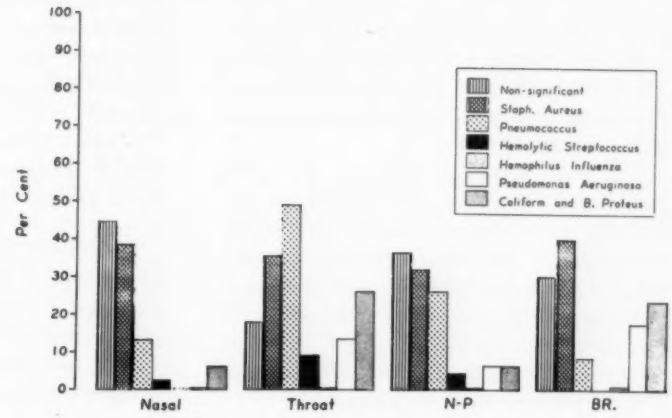

Fig. 4. Results of cultures obtained simultaneously from four sites of the respiratory tract of non-CF patients.

Staph. aureus in CF patients was not limited to those with an advanced clinical state. Of the 10 CF patients who have died in the past 4 years 6 harbored type 80/81; the remaining 4 had other phage types, namely, 7/77, 47/75, 44 A, and 77, respectively.

ANTIBIOGRAM OF STAPHYLOCOCCUS AUREUS

A total of 101 strains of *Staph. aureus* isolated from the bronchial secretions and 107 strains isolated from the swabs of the nasopharynx of Groups II and III CF patients were tested for susceptibility to 12 antibiotics by the tube-dilution method (Table V). The number of strains of *Staph. aureus* obtained from non-CF patients similarly tested was too small to be included for comparison. The results indicated that strains of the 80/81 types of *Staph. aureus* isolated from both nasopharyngeal and bronchial secretions of CF patients were more resistant to penicillin, streptomycin, and tetracycline than were other strains, but the difference is only significant to the 6 per cent level by statistical analysis. No striking differences were found in the susceptibility patterns of type 80/81, other phage types, and nontypable *Staph. aureus* to the other antibiotics. All strains of *Staph. aureus* were highly susceptible to bacitracin, ristocetin, vancomycin, and novobiocin, and moderately susceptible to neomycin, oleandomycin, erythromycin, chloramphenicol, and kanamycin in decreasing order. The majority

Table VI. *Pseudomonas aeruginosa* inhibited by 7 antibiotics in quantities of 15.6 mcg. per milliliter or less as determined by tube-dilution method

Antibiotic agent	*Nasopharyngeal*		*Bronchial*	
	No. tested	*Sensitive (%)*	*No. tested*	*Sensitive (%)*
Chloramphenicol	23	4	45	17
Kanamycin	17	12	23	0
Neomycin	23	43	45	31
Polymyxin	23	58	47	38
Streptomycin	23	17	46	11
Tetracycline	23	30	42	21
Oxytetracycline	23	35	47	43

of the strains tested were isolated between 1958 and 1960. No appreciable changes in susceptibility patterns have been observed in the strains isolated in 1958 as compared to those isolated in 1960.

The susceptibility patterns of *Ps. aeruginosa* to 7 antibiotics determined by the tube-dilution method are shown in Table VI. It is apparent that *Ps. aeruginosa* is a highly resistant organism. Among the antibiotics tested, polymyxin, neomycin, and oxytetracycline were effective to some degree, whereas chloramphenicol, kanamycin, streptomycin, and tetracycline were ineffective.

DISCUSSION

The results of this investigation indicate the presence of a much higher incidence of bacterial pathogens in the throat, nasopharyngeal, and bronchial secretions of CF patients than of non-CF patients. By contrast, non-CF patients showed a higher frequency of nonpathogenic organisms. The prevalence of *Ps. aeruginosa* is much greater in the bronchial, throat, nasopharyngeal, and nasal cultures of CF patients; coagulase-positive *Staph. aureus* were more frequent in the throat, nasopharyngeal, and bronchial cultures of CF patients than of non-CF patients. This difference was most striking in bronchial and throat cultures. Thus, *Staph. aureus* and/or *Ps. aeruginosa* constitute the principal flora of the respiratory tract of CF patients, whereas no constant bacterial pattern has been observed in non-CF patients.

In the non-CF patients, throat cultures yielded the most bacterial pathogens. In non-CF patients *Staph. aureus* was isolated from all parts of the respiratory tract, but its incidence in throat and bronchial specimens was significantly lower than in CF patients. However, the nasal carrier rate of coagulase-positive *Staph. aureus* by non-CF patients is higher than that of CF patients, but the difference is not statistically significant.

The importance of previous antibiotic therapy in the establishment of the respiratory flora is shown by the data in Table VII. Most CF patients had a history of repeated administration of antibiotics prior to admission to the hospital. The predominant flora of those who had received antibiotic therapy before admission consisted mainly of *Staph. aureus* and/or *Ps. aeruginosa,* whereas those without prior antibiotic therapy harbored a flora very similar to that of non-CF patients.

The results of this study showed that over 90 per cent of all throat and bronchial cultures of CF patients were positive for bacterial pathogens, whereas 50 to 80 per cent of nasal cultures and 65 to 80 per cent of nasopharyngeal cultures yielded pathogenic

Table VII. Relationship of flora of the respiratory tract obtained on admission to previous antibiotic therapy of CF patients

Bacterial pattern of flora	*Previous antibiotic therapy*			
	Positive		*Negative*	
	No.	*%*	*No.*	*%*
Nonsignificant	0	0	5	21.7
Staph. aureus	18	34.6	5	21.7
Ps. aeruginosa	8	15.4	2	8.7
Coliform organism	2	3.8	5	21.7
Staph. aureus and *Ps. aeruginosa*	18	34.6	1	4.4
Staph. aureus and coliform	5	9.7	0	0
Pneumococcus and/or *H. influenzae*	1	1.9	5	21.7
Total	52		23	

Table VIII. The predominant flora of the respiratory tract of CF patients and its relationship to the severity of respiratory infection

Severity of respiratory infection*	No. of patients	Predominant flora of the respiratory tract						
		Staph. aureus	Ps. aerug.	Coliform or B. proteus	Staph. aureus + coliform or B. proteus	Staph. aureus + Ps. aeruginosa	Miscellaneous	Non-significant
Mild	22	9	1	0	1	6	0	5
Moderate	19	11	3	0	0	4	0	1
Advanced	67	13	11	3	10	29	1	0

*Mild, roentgenologic evidence of overaeration.
Moderate, roentgenologic evidence of overaeration and peribronchial infiltration.
Advanced, roentgenologic evidence of overaeration, peribronchial infiltration, and/or atelectasis, and/or bronchopneumonia.

organisms. Except for the increased isolation of the pneumococcus in throat cultures the incidence of bacterial pathogens in the throat was similar to that in the nasopharynx. Bronchial cultures would appear, therefore, to be more representative of the organisms involved in the lower respiratory tract than would nasopharyngeal or throat cultures. However, in patients with advanced pulmonary lesions, throat and nasopharyngeal secretions are equivalent to bronchial secretions for the isolation of respiratory pathogens. The relationship of the flora of the respiratory tract to the severity of pulmonary infection is shown in Table VIII. The predominance of *Staph. aureus* and *Ps. aeruginosa* in patients with advanced pulmonary lesions is apparent. Among the 32 CF patients who died during the period of observation, 18 had *Staph. aureus* and *Ps. aeruginosa* in their bronchial cultures; 7 of the group had *Staph. aureus* alone or in combination with Proteus or coliform organisms; the remaining 7 patients had *Ps. aeruginosa* or coliform organisms or combinations of these and other gram-negative bacilli. In most CF patients *Staph. aureus* was the first pathogen to become established in the respiratory tract followed by *Ps. aeruginosa* after a prolonged period of therapy.

The danger of converting a susceptible bacterial flora to a highly resistant one by antibiotic therapy is apparent.

Most non-CF patients in this series also received antibiotic therapy intermittently. Eighteen of 30 non-CF patients who had continuous administration of antibiotics for prolonged periods of time ranging from several months to 2 or 3 years for chronic bronchitis, bronchiectasis, and recurrent pneumonitis harbored *Staph. aureus* in their bronchial secretion; 6 of them had *Ps. aeruginosa* in the bronchial culture. However, the combination of *Staph. aureus* and *Ps. aeruginosa* occurred in 3 patients only occasionally. Therefore, antibiotic therapy alone cannot explain the persistence of *Staph. aureus* and *Ps. aeruginosa* in the respiratory tract of CF patients. Bronchial obstruction caused by very tenacious bronchial mucus of CF patients is another important factor favoring the localization of bacterial pathogens in the respiratory tract.

In respect to the local defensive mechanisms of CF patients, it is of interest that there was no difference in the number of plasma cells in the bronchial secretion of CF patients as compared to that of non-CF patients.[13] Green[14] and his group found the lysozyme levels normal in the saliva and elevated in the gastric juice and duodenal fluid of CF patients. The increased lysozyme content of the gastric and duodenal fluids was thought to be related to the swallowed sputum which might have increased the lysozyme content.

The immune mechanism of CF patients was tested by immunizing a number of them with Vi antigen and polyvalent influenza virus vaccine.[15] The results showed that CF patients responded with antibody formation as well as normal children. This is in agreement with the findings of Eichenwald.[16]

No systematic study has been carried out concerning the incidence of viral infections in CF patients, but careful clinical observation has not indicated an increased incidence of viral infections in the CF patients as compared with the non-CF. However, as Eichenwald[16] has pointed out, even the average individual incidence of infections by respiratory viruses may disrupt the local defensive mechanisms of CF patients and favor bacterial multiplication. Eichenwald[17] postulated bacterial-viral synergism in newborn infants. There is clinical evidence that CF patients frequently become worse following an attack of measles or influenza. Such a response may follow infections with other common respiratory viruses.

No differences have been observed in the phage types of *Staph, aureus* isolated from CF patients as compared with those of non-CF patients. These findings are in agreement with those of Pittman and co-workers[18] and suggest that these may be strains acquired during hospitalization. *Staph. aureus* has been repeatedly isolated from random cultures of the skin and bedding of CF patients with advanced lesions. However, the staphylococcal infection has usually been limited to the respiratory tract of these patients, except for infants under 6 months of age. In the latter age range, we have seen CF patients with otitis media and empyema due to *Staph. aureus.*

The highly resistant nature of the bacterial flora of CF patients is apparent from the results of antibiotic susceptibility tests. Management of these patients should be highly individualized according to the antibiograms of their pathogenic flora. In some instances, when the pathogens were highly resistant in vitro to all of the available agents, chloramphenicol has been found to be effective against both *Staph. aureus* and *Ps. aeruginosa* in spite of the in vitro resistance of the organisms.

SUMMARY

1. Bacterial flora of the upper and lower respiratory tract of patients with cystic fibrosis of the pancreas and those with a chronic respiratory tract of patients with cystic fibrocauses have been studied.

2. The results showed a markedly increased incidence of *Staph. aureus* in the throat and bronchial secretions, and of *Ps. aeruginosa* in cultures obtained from all the sites of the respiratory tract of CF patients.

3. In non-CF patients there was a significantly lower incidence of cultures yielding pathogens. Pneumococci and *H. influenzae* were isolated more frequently in non-CF patients than in CF patients.

4. A total of 251 strains of *Staph. aureus* which were isolated from nasopharyngeal and bronchial secretions of CF and non-CF patients were tested for their bacteriophage patterns. The results showed no difference in the distribution of phage type of *Staph. aureus* isolated from the 2 groups of patients.

5. Antibiograms of a total of 101 strains of *Staph. aureus* from the bronchial secretions and 109 strains from the nasopharyngeal secretions of CF patients were determined against 12 antibiotics. No significant difference was found in the antibiotic susceptibility pattern of the different phage types tested except that the type 80/81 strains were highly resistant to penicillin, streptomycin, and tetracycline.

6. All the strains of *Staph. aureus* tested were highly susceptible to bacitracin, ristocetin, vancomycin, and novobiocin, and fairly susceptible to neomycin, oleandomycin, erythromycin, chloramphenicol, and kanamycin, in decreasing order. The antibiotic susceptibility patterns of a total of 70 strains of *Ps. aeruginosa* against 7 antibiotics showed that these organisms were highly resistant to the antibiotics tested. Only polymyxin, neomycin, and oxytetracycline were fairly effective against some strains.

We wish to express our gratitude to Dr. Kenneth M. Schreck for performing the bacteriophage typing of *Staphylococcus aureus,* to Professor Earle H. Spaulding for his most invaluable general advice, and to Miss Hilda Spence, Mrs. Edith Cheng, and the staff of the diagnostic bacteriology laboratory for their technical assistance.

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Achalasia in children as a cause of recurrent pulmonary disease

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THE clinical problem of recurrent, nonspecific pulmonary infections in children often leads to a search for an underlying systemic disorder such as cystic fibrosis, agammaglobulinemia, or familial dysautonomia. Bronchoscopy and bronchography may be undertaken to determine possible structural abnormality. The sinuses and tonsils are viewed with suspicion.

It is the purpose of this paper to report 2 cases of achalasia of the esophagus associated with chronic respiratory problems in children. Achalasia is not a rare disorder in adults, but it is seldom considered in pediatric patients. In children the recurrent respiratory symptoms due to aspiration often overshadow any dysphagia, and the child may not complain at all of the latter.

REPORT OF CASES

Case 1. A 10-year-old white female child was seen by her physician with a complaint of gurgling noises in the throat which had been noted at night for 2 years. These noises were sometimes accompanied by an overflow of liquid from the mouth and were almost always followed within a few hours by cough, chills, and fever. During this time there had been difficulty with swallowing solid foods, relieved in part by taking liquids or by digital pressure against the suprasternal notch. She occasionally forced herself to retch, and then could complete her meal without difficulty.

Past history revealed an episode of pneumonia at 18 months of age, and very frequent "colds" throughout her life.

Physical examination revealed a thin child in no obvious distress. No abnormality was found on examination of the mouth and throat or on general physical examination. The lungs were clear to auscultation. Laboratory studies were not remarkable except for a mild normochromic anemia.

X-ray examination of the chest showed no localized consolidation, but there was a bilateral generalized increase in pulmonary markings consistent with mild, diffuse interstitial reaction secondary to repeated aspirations (Fig. 1). Fluoroscopic examination and roentgenogram of the esophagus with barium showed typical changes of achalasia (Fig. 2).

Dilatation of the distal esophagus was subsequently performed several times with

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the use of a Mosher bag. Although symptoms occasionally recurred, they were less severe and the child was considerably improved during the next 3½ years. Occasional recurrences of dysphagia and night cough were promptly relieved by dilatations. The esophagus returned toward a more normal size.

Case 2. An 8-year-old white male was seen at the University of Florida Teaching Hospital with a chief complaint of fever and nausea of 4 months' duration.

One year previously the child had had pain in the left side of the chest and fever, which were treated with penicillin; recovery was complete within a week. No joint complaint or other disturbance was evident.

Six months before admission, the child began to lose his appetite after taking a few bites of food at each meal. Two months later recurrent nausea and an uncomfortable sensation in the epigastric region became evident. His mother subsequently noted a brown, fluid material on his pillow each morning, and he would spit up similar material at intervals during the day. Beginning at this time the mother noted a recurrent, low-grade fever, without chills or night sweats. For several days before being seen, he had had a nonproductive cough associated with a rectal temperature up to 102° F. The past history revealed normal growth and development up to the time of the present illness. The usual immunizations had been done. The child had had measles, chickenpox, and mumps without sequelae. A review of symptoms by organ systems was noncontributory. Both the parents were living and well, and there was no history of a similar illness in the family.

On physical examination, the vital signs were normal. Height was measured 52½ inches (fiftieth percentile), and weight 56 pounds (seventeenth percentile). General physical examination was within normal limits. The lungs were clear to percussion and auscultation, and there was no unusual odor to the breath.

Urinalysis was not remarkable. Examination of the peripheral blood showed hemoglobin of 12.7 Gm. per cent, hematocrit of 38 per cent, and white blood cell count of 10,050 per cubic millimeter, with a normal differential cell count except for 6 per cent eosinophils.

The patient was referred for radiologic study. X-ray of the chest (Figs. 3 and 4) revealed the right lung to be clear. At the base of the left lung there were interstitial infiltrations with faintly increased density, crowding of vascular markings, and loss of the sharp normal shadow of the adjacent diaphragm, which are consistent with a subsiding pneumonitis.

Examination of the upper gastrointestinal tract was done with barium. The esophagus was moderately dilated, and no peristaltic waves were seen. The lumen of the esophagus

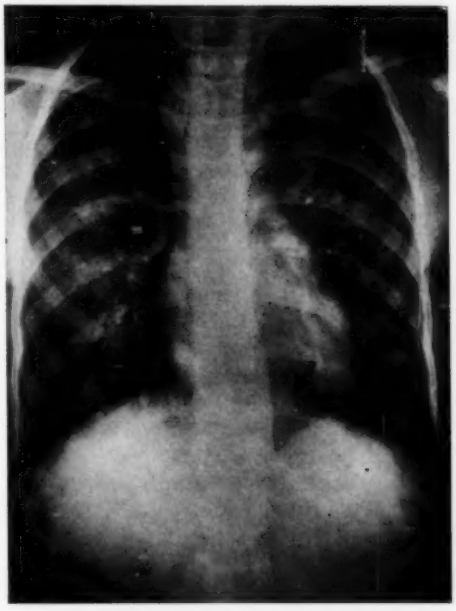

Fig. 1. Posteroanterior view of the chest in Case 1 reveals a diffuse increase in interstitial markings throughout the lungs, consistent with chronic interstitial reaction secondary to recurrent aspiration. The right edge of the dilated esophagus in the superior mediastinum is indicated by the upper arrows. The lower arrows indicate the margin of the dilated esophagus just above the diaphragm, behind the heart. Note the absence of the usual gas bubble in the stomach.

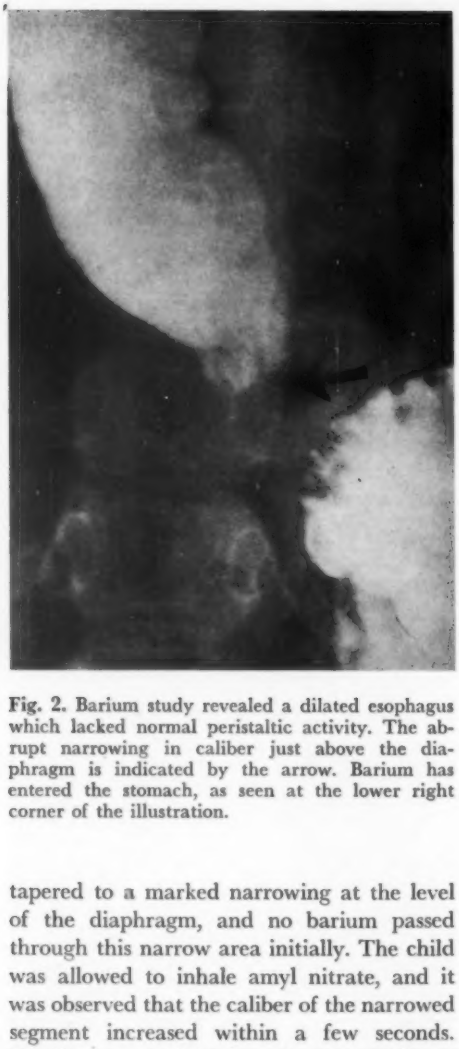

Fig. 2. Barium study revealed a dilated esophagus which lacked normal peristaltic activity. The abrupt narrowing in caliber just above the diaphragm is indicated by the arrow. Barium has entered the stomach, as seen at the lower right corner of the illustration.

tapered to a marked narrowing at the level of the diaphragm, and no barium passed through this narrow area initially. The child was allowed to inhale amyl nitrate, and it was observed that the caliber of the narrowed segment increased within a few seconds. Small amounts of barium then passed intermittently into the stomach (Fig. 5). The stomach and duodenum could not be examined adequately because of the small amount of barium that entered these organs. A diagnosis of achalasia was made.

Subsequently, esophagoscopy and a Heller myotomy were performed under general anesthesia; the mucosa appeared normal at the area of esophageal narrowing, without evidence of chronic inflammation. Longitudinal incision of the distal esophagus and gastric fundus, down to the mucosa, was done. The child was discharged 7 days later after an uncomplicated convalescence.

At the last clinic visit, 17 months later, there had been no recurrence of fever, nausea, regurgitation, or difficulty with eating. Radiologic examination of the esophagus at this time revealed prompt passage of barium through the esophagus in upright position. In supine position emptying of the esophagus occurred slowly, but there was no local point of obstruction. No coordinated peristaltic activity was evident, although intermittent contractions occurred. The esophagus remained slightly dilated (Fig. 6).

PATHOLOGY

In patients with achalasia, there is partial and apparently functional obstruction to emptying of the esophagus, centered near the esophagogastric junction. Early in the disease, the esophagus dilates only slightly and hypertrophy of circular muscle fibers occurs, preventing retention of contents in the esophagus.[1, 2] With continued obstruction the esophagus becomes progressively dilated. On x-ray examination and at postmortem there is regularly seen a short, undilated segment, 2 to 4 cm. in length, in the most distal portion of the esophagus.[1, 3, 4]

In adults, after many years of symptoms, the esophagus degenerates to a markedly distended, elongated, and tortuous tube, continuously retaining a liter or more of food and secretions. In children, the esophagus usually has been found to be only moderately dilated, which is consistent with the shorter duration of the disease.

Microscopic examination reveals degeneration, necrosis, and fibrotic replacement of nerve ganglion cells in the myenteric plexus of the esophagus. First emphasized by Hurst and Rake,[2] this pathologic basis for the physiologic disturbance has been confirmed by most subsequent investigators.[1, 3, 4] The reduction of ganglion cells is most marked in the distal esophagus, including the undilated segment.[2] There is no fibrosis or stricture in the undilated segment. While some muscular hypertrophy may be found, it will also then be present in the dilated

portion of the esophagus as well.[1] Sections taken just below the undilated segment show normal numbers of ganglion cells.[3]

Freezing[5] or injection of phenic acid[6] into the distal esophagus in animals produced degeneration and necrosis of ganglion cells with reproduction of the radiologic and postmortem appearance of achalasia.

Studies of intraluminal pressures in the distal esophagus in response to drugs have shown responses characteristic of denervated intestine in patients with achalasia. Such response has not been obtained in patients with esophageal obstruction caused by strictures or tumors.[7, 8]

The evidence indicates that the disease is due to disorganization and perhaps ablation of effective parasympathetic stimuli to the distal esophagus, with failure of relaxation (achalasia) of the segment of normal high tone near the esophagogastric junction.[2, 4, 8] There is little evidence to support the older theory of primary spastic contracture at this level (cardiospasm), although the terms are often used interchangeably.

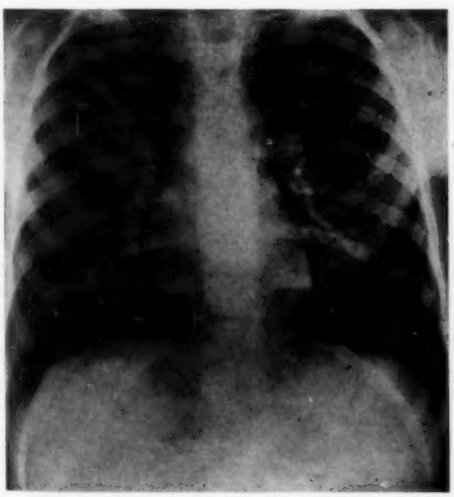

Fig. 3. In Case 2, the esophagus has not dilated sufficiently to be visible on posteroanterior view of the chest. There is a patchy area of consolidation at the base of the left lung, as described in the text. The normal gastric air bubble is not present below the diaphragm.

Fig. 4. Detail of the consolidation at the left base noted in Fig. 3, with blurring of the normally sharp diaphragm shadow, is shown.

CLINICAL FEATURES

Symptomatic achalasia is usually encountered in adults. Of 691 consecutive cases of achalasia reported from the Mayo Clinic, 5 per cent of the patients had symptoms which began before the age of 14 years.[9] A later, similarly large series showed an incidence of 3 per cent with symptoms starting in the first decade of life.[10]

Several instances of recurrent vomiting in the immediate postnatal period have been reported as cases of "cardiospasm."[11-15] The diagnosis is based on radiologic demonstration of a slightly dilated esophagus and the presence of contrast medium in the esophagus for several hours. These infants were all relieved by a few days of gastric intubation or by the administration of atropine, and had no further difficulty.

In older children, the insidious onset of dysphagia is probably the most common presenting symptom.[9] A sensation of substernal discomfort or actual pain may occur in adults but is rarely mentioned in the reported cases in children. Occasionally the

Fig. 5. Barium study of the esophagus in Case 2 shows the elongated narrow segment at the level of the diaphragm, indicated by the arrow. The esophagus above this segment is moderately dilated. This film was made after inhalation of amyl nitrate, and some barium has entered the stomach.

child feels a sense of obstruction in the throat, with the result that tonsillectomy is performed for relief.[9]

A number of case reports emphasize that these children may appear at first to present problems of chronic lung disease with cough, sputum production, and recurrent episodes of pneumonia.[16-20] Bronchiectasis has been frequently mentioned as the first clinical impression in these cases, and it indeed occurs in untreated cases.[17, 19] Belcher reviewed the clinical features of 6 teen-age patients with achalasia and significant chronic pulmonary disease; chronic cough was the chief complaint in 5 of these children, and dysphagia was a prominent symptom in only one.[21]

All patients with achalasia are potential candidates for pulmonary disease; significant involvement is present in about 10 per cent of adults with achalasia.[22, 23] A large series of consecutive cases of achalasia in children has not been reported, but from various case reports the incidence of pulmonary involvement would seem greater than in adults.

Since the fluid level in the dilated esophagus does not reach the level of the glottis in the erect position, overflow and aspiration probably occur primarily during sleep.[24] Nocturnal regurgitation,[25] recurrent cough and episodes of pneumonia are prominent in the history. Areas of chronic pulmonary infiltration and fibrosis are the most common radiologic finding in the cases with pulmonary disease,[26] but bronchiectasis, lung abscess, and lobar atelectasis occur.[9, 21-23] Sudden death by aspiration of esophageal contents, while bending over, has been reported.[27]

It is of interest that nonpathogenic, acid-fast, apparently saprophytic bacilli can be recovered from the sputum or gastric washings from a significant number of patients with achalasia and pulmonary complications.[23, 26, 28] The possibility of confusion of the chronic pulmonary manifestations with tuberculosis is evident. Indeed, the recurrent aspiration of milk fats floating at the top of the esophageal contents probably enhances survival and proliferation of mycobacteria, allowing saprophytic strains to became actively pathogenic.[28]

RADIOLOGIC FEATURES

The diagnosis of achalasia is based upon radiologic demonstration of the characteristic anatomic and physiologic disturbances of

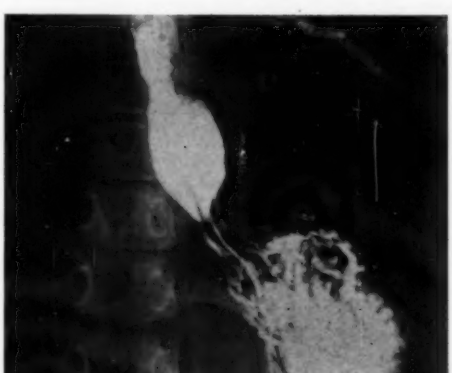

Fig. 6. Following a Heller procedure, barium examination of the esophagus shows a more normal caliber at the esophagogastric junction (arrow), and barium passed promptly from the esophagus into the stomach. The esophagus showed some contractions, but no organized peristalsis was present. The emptying of the esophagus was prompt in the upright position.

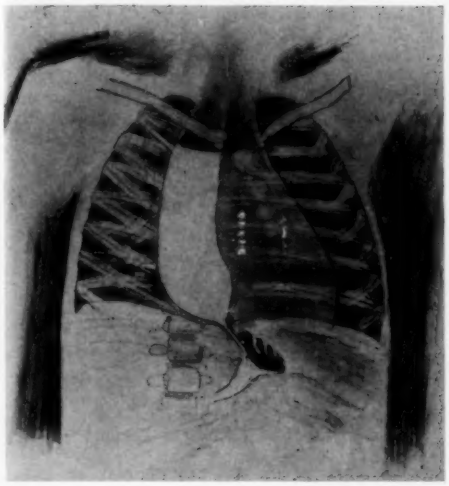

Fig. 7. A drawing is shown illustrating the typical anatomic changes in achalasia, as would be revealed by radiologic examination in oblique projection. The dilated esophagus is filled with barium and secretions, with an air-fluid line near the level of the clavicle. The abrupt taper to a narrowed segment at the level of the diaphragm is shown. A small quantity of barium has entered the stomach.

the esophagus. On plain films of the chest the wide, fluid-filled esophagus sometimes can be seen in the posterior mediastinum, as in Case 1. Indeed, one child with achalasia was treated with x-ray therapy for a posterior mediastinal "mass" before the correct diagnosis was made.[16] An air-fluid level often is evident, high in the posterior mediastinum. The air bubble in the stomach, almost always seen in normal children because of air swallowing, is often absent.

In children, barium study usually reveals the esophagus to be moderately dilated (Fig. 7). The ingested barium mixes with variable amounts of retained secretions in the esophagus. The tapered narrowing of the esophagus at the level of the diaphragm, as well as the long delay before any barium enters the stomach, can be demonstrated. After ingestion of barium the esophagus may show some random contractions, but a self-propelling, normal peristaltic wave is not seen.

Differentiation must be made from organic stricture of the distal esophagus, for which fluoroscopic observation is essential. In patients with stricture, normal or hyperactive peristaltic waves usually can be detected passing the entire length of the thoracic esophagus. After inhalation of amyl nitrate, the caliber of the tapered narrowing will increase in patients with achalasia, allowing a variable quantity of contrast medium to enter the stomach. The caliber of an organic stricture will be unaffected by this procedure.

DISCUSSION

Children with partial obstruction of the esophagus may fail to express their sensation of dysphagia, as in Case 2, and younger children may accept it as normal. In children with recurrent pulmonary infections of obscure origin, barium study of the esophagus is a simple procedure which may disclose a localized anatomic abnormality amenable to surgical correction. Certainly a barium swallow should precede bronchoscopy or other surgical diagnostic procedures.

Since nonpathogenic acid-fast bacilli have been recovered from many children with achalasia, study of the esophagus with barium would seem reasonable in any child from whom acid-fast bacilli are recovered in the presence of a negative skin test for tuberculosis.

Both achalasia of the esophagus and Hirschsprung's disease of the large bowel are secondary to loss of ganglion cells in a portion of their walls. The defect is congenital in megacolon; in contrast, the histologic studies from patients with achalasia suggest an inflammatory or degenerative process replacing ganglion cells previously present. This correlates with the usual clinical onset of achalasia in adulthood.

The disorder that has been reported as "cardiospasm" in the neonatal period apparently does not represent the same pathologic condition as achalasia in older children and adults, since prompt recovery usually occurs within a few days. On barium study the esophagus of normal infants often is wide in relation to the chest when compared to standards in adults, and, in infants in the

supine position, emptying into the stomach is normally delayed for variable periods. The detection of barium in the esophagus of infants for several hours, by x-ray film study, is as likely due to intermittent reflux from the stomach as to retention in the esophagus. It seems doubtful that the true pathologic entity of achalasia has ever been observed in infancy. Vinson[29] believes that transient delay in the emptying of the esophagus is frequent at birth, and that no therapy is necessary or helpful.

SUMMARY

Achalasia of the esophagus in children may be manifest only by recurrent pulmonary infections secondary to aspiration.

The disorder represents one of the more easily remediable causes of chronic respiratory disorders in children.

Two cases of achalasia in children with pulmonary complications are reported.

I wish to thank Drs. Allen DeLaney and Maxey Dell for permission to study their patient, Case 1.

Dr. B. G. Brogdon kindly supplied the drawing for Fig. 7.

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Accessory diaphragm associated with neonatal respiratory distress

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IT IS the purpose of this brief report to call attention to a rare anomaly consisting of partitioning of the right hemithorax into 2 compartments by a muscular and tendinous membrane. This membrane may interfere with respiration, causing respiratory distress in the neonatal period or chronic pulmonary inflammation in later life.

CASE REPORT

This white infant boy, who lived less than 5 hours, was transferred to this hospital with the following history. He was born 4 weeks prematurely, birth weight 4 pounds, 7 ounces. Except for the early labor, pregnancy had been uneventful. Delivery was without instruments. He cried spontaneously but was noted to be very mildly cyanotic in the delivery room. Twenty minutes after delivery, when in the nursery, he was seen to be deeply cyanotic. Mechanical respiration was attempted by giving oxygen with a mask, pressure being controlled by an E & J resuscitator. His color did not improve, cyanosis persisted, and grunting respirations began. A chest film revealed pneumothorax on the left side, the lung lying ½ inch from the chest wall.

On arrival here, he was cyanotic and deceptively quiet. Respirations were 48 per minute and without retraction or flaring of nostrils. Breathing was predominately right-sided, the left chest lagging and moving only slightly. Breath sounds were absent on the left side and diminished on the right. The heart rate was regular and 160 per minute. There were no murmurs, and the sounds were heard best to the right of the sternum. The pupils were dilated and reacted only slightly to light.

A chest film revealed left pneumothorax with mediastinal displacement to the right.

Breathing became more shallow and the cyanosis continued in spite of the insertion of an endotracheal tube and aspiration of 30 c.c. of air from the left chest. He died 1 hour and 15 minutes after admission.

Autopsy findings. The mechanism of death was interpreted as asphyxia due to collapse of the lungs secondary to left pneumothorax

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Table I. Summary of reported cases

Author	*Age*	*Sex*	*Neonatal symptoms*	*Later symptoms and signs*	*Operation*
Sullivan[3]	4 years	M	Cyanosis requiring oxygen first 24 hours	Cough, fever, repeated pneumonia for 2 years	Partial resection of accessory diaphragm
Sappington and Daniel[2]	7 years	F	Not stated	Cough and hemoptysis for 1 month Mediastinal shift to right	Resection of accessory diaphragm
Drake and Lynch[1]	24 years	M	Not stated	Cough with copious sputum for 5 years One episode of hemoptysis Dyspnea	Right lower lobectomy Resection of accessory diaphragm
Present case	1 day	M	Cyanosis at birth		

and an anomalous septum in the right thoracic space.

Findings of interest were confined to the thorax. Pneumothorax was demonstrated on the left side by opening this cavity under water. The left lung was partially collapsed but contained some air in the periphery. Disseminated interstitial emphysema in this lung was continuous with emphysema of the mediastinum. The mediastinum was shifted to the right so that the left border of the heart was retrosternal.

The right thoracic cavity was divided by a fibromuscular membrane attached laterally and posteriorly along the course of the seventh rib. Anteriorly and medially it lay just above the normal right diaphragmatic leaf and on the pericardium near its diaphragmatic attachment. The upper and lower right thoracic cavities made by this membrane were incompletely divided as the 2 spaces communicated through a semicircular orifice, 2.0 cm. in diameter. The base of this hiatus was on the posterior mediastinum and the bronchi and vessels to the middle and lower lobes passed through it to these lobes which lay in the inferior cavity between the accessory and true diaphragms. The accessory diaphragm was transparent in its periphery, but near the smoothly lined hiatus thin strands of muscle were arranged in curving bands that thinned toward the periphery. Both upper and lower surfaces of this anomalous septum were continuous with the parietal pleura lining the chest wall of the 2 cavities. The middle and lower lobes were easily delivered superiorly through the orifice (Figs. 1 and 2). The inferior surface of the lower right thoracic cavity was formed by a normal right diaphragmatic leaf.

The lungs, heart, and great afferent and efferent vessels were normally formed, and the division of the right hemithorax by the anomalous membrane was the only

Follow-up	Composition of accessory diaphragm	Location of accessory diaphragm	Contents of lower compartment	Associated anomalies
Thriving 1 year postoperatively	Striated muscle and tendon	Right side, curved over and compressed pulmonary artery and bronchus Blended with true diaphragm anteriorly	Right, middle, and lower lobes	Agenesis of right upper lobe
Little motion of right diaphragm 2 months postoperatively	Striated muscle and tendon Received branch of phrenic nerve	Right side, attached anteriorly to pericardium and central tendon Arched posteriorly at level of fifth rib 4 cm. hiatus near pericardium	Right lower lobe	Incomplete fissure right upper and middle lobes Hypoplasia of normally placed right leaf
Improved, some cough persisting 1 year	Striated muscle and tendon	Right side, arose from dome of normal right leaf and extended posteriorly to lie 2 inches above normal diaphragm	Lower segment of lower lobe	Incomplete fissure right upper and middle lobes Inferior right pulmonary vein draining to inferior vena cava passed through normal diaphragm
Death prior to operation	Striated muscle and tendon	Right side, joined pericardium and normal right leaf anteriorly Followed course of seventh rib 2 cm. hiatus posteromedial	Right, middle, and lower lobes	None

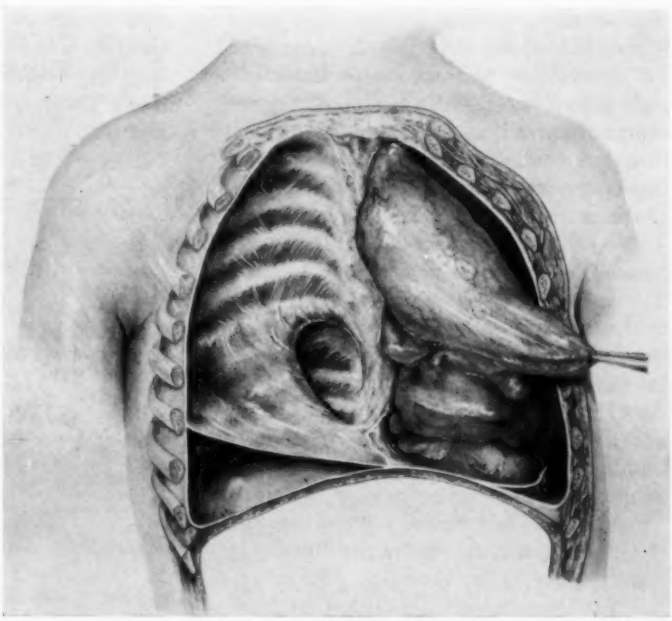

Fig. 1. In this sketch, the middle and lower lobes have been delivered through the hiatus in the accessory diaphragm, and the entire right lung has been retracted medially. The position of the accessory diaphragm is demonstrated.

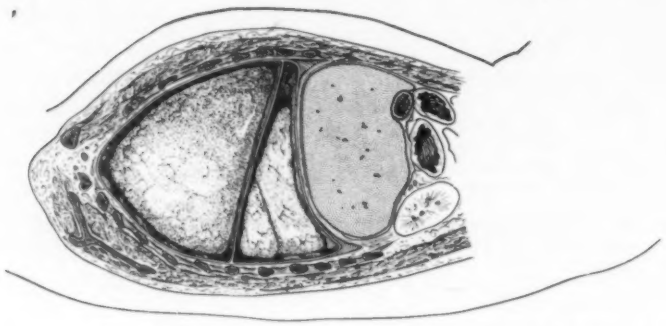

Fig. 2. A paramedian section shows the contents of the 2 right thoracic cavities.

gross or microscopic congenital anomaly in this case.

Abnormal microscopic findings were confined to the lungs, and were of atelectasis or lack of expansion.

DISCUSSION

A search of medical literature for the past 33 years revealed 3 reported cases of accessory diaphragm.[1, 2, 3] These cases with the above case are summarized in Table I. From these observations, it can be stated that the anomaly of accessory diaphragm consists of partitioning of the right hemithorax into upper and lower compartments by a muscular and tendinous diaphragm with a posteromedial hiatus connecting the compartments. Pressure at the hiatus and disturbed mechanics due to the accessory diaphragm interfere with function, especially of the contents of the inferior compartment. These contents may consist of all, or portions of, the right, middle, and lower lobes. The level of the posterolateral attachment of the accessory leaf to the chest wall varies from case to case altering the size of the inferior cavity. Anomalies of lobation of the right lung may accompany this anomaly, and anomalous pulmonary venous connection was noted in one case. The normally placed right diaphragmatic leaf was reported as normally formed in 3 instances and as hypoplastic in the other.

Clinically, accessory diaphragm may be associated with respiratory difficulty at birth. If the neonatal period is survived, symptoms of chronic pulmonary infection may appear later. This lesion is amenable to surgical therapy.

SUMMARY

A case of accessory diaphragm associated with respiratory difficulty at birth is described. Three similar cases, summarized from the literature, show that this anomaly is not always lethal in the neonatal period, and that it may be associated with chronic pulmonary infection in later life.

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Anemia associated with protein deficiency

A study of 2 cases with cystic fibrosis

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IT IS generally accepted that very low levels of protein in the diet of growing animals lead to hypoproteinemia. It is less well known that this may also lead to anemia of moderate degree.[1-5] In addition Cartwright and Wintrobe[1] have shown that, in swine, the hypoproteinemia is associated with hypocupremia, hypoferremia, and a reduction of the total iron-binding capacity of the serum. In human beings, anemia and similar biologic abnormalities in association with an inadequate intake of protein have been described in children with kwashiorkor.[6] However, the presence of infection[7] and the deficiencies of folic acid[8] and other essential food factors in many of these patients may interfere with the evaluation of these biologic alterations and of the quantitative defect in hemoglobin synthesis resulting from protein deficiency.

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In 1955 Bille and Vahlquist[9] indicated that protein deficiency, resulting in edema and hypoproteinemia, may occur in infants and children with pancreatic fibrosis, presumably caused by inadequate digestion and absorption of dietary protein. Four patients with such a condition were recently admitted to the medical ward of the Boston Children's Hospital, and we studied two of them in some detail. The present report will describe, in addition to hypoproteinemia and anemia, the results of studies on serum iron, iron-binding globulin, and serum copper in these 2 patients with emphasis on the effect of protein deficiency on hemoglobin synthesis.

MATERIALS AND METHODS

The cases to be reported are of 2 infants admitted for diagnosis and treatment of edema and found to have cystic fibrosis of the pancreas. The diagnosis was made by aspiration and analysis of duodenal fluid and by analysis of sweat induced by the method described by Shwachman and co-workers.[10]

Detailed hematologic studies were carried out by the following techniques: Blood counts were estimated by conventional he-

Fig. 1. Showing the pitting edema of the lower extremity.

matologic methods; the concentration of total protein in the serum was determined by the micro-Kjeldhal method.[11]

Electrophoretic analysis of the serum proteins was performed by the Spinco Method R.[12] The concentration of protein fractions on paper strips was measured with the Spinco Analytrol.*

The method employed for the measurement of serum iron has been described by Barkan and Walker[13] and that for total iron-binding capacity by Cartwright and Wintrobe.[14] The serum copper was measured by the method described by Eden and Green.[15] The concentration of β_1-metal-binding globulin was determined immunochemically with the use of anti-β_1-globulin rabbit serum according to the method described by Gitlin.[16] Albumin turnover studies were performed with albumin labeled with I^{131} following the method described by Sterling.[17]

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CASE REPORTS

Patient 1. A 2-month-old male infant was admitted for the first time to the Children's Hospital Medical Center on March 16, 1958, because of generalized edema and pallor. The infant was the first born of a healthy mother following a normal pregnancy. The birth weight was 8 pounds, 9 ounces. The neonatal period had been uneventful. He was breast fed for the first week and then given an evaporated milk formula supplemented with vitamins A, C, and D. Because of frequent stools and the appearance of a papulo-erythematous eruption at the age of 5 weeks, a diagnosis of "milk allergy" was considered and a soya bean protein preparation (Sobee) was substituted for the evaporated milk formula. Despite an excellent appetite, the gain in weight was not satisfactory. Two days prior to admission the patient was noticed to have swelling of both lower extremities.

Physical examination on admission revealed an edematous, moderately pale infant in no distress. He weighed 9 pounds, 15 ounces, and appeared to be well developed. The temperature was 99° F. and pulse rate 100 per minute. Edema was generalized and marked pitting was demonstrable (Fig. 1). There was no enlargement of the liver or spleen.

Laboratory data. Examination of the urine on several occasions showed no abnormality. Total serum protein on admission was 3.2 Gm. per cent, with an albumin fraction of 1.5 Gm. per cent. Hemoglobin concentration was 7.2 Gm. per cent; the stained film of the peripheral blood revealed normochromic normocytic erythrocytes and an adequate number of platelets. There were 11,600 white blood cells per cubic millimeter, with a differential count of 26 per cent polymorphonuclear leukocytes, 62 per cent lymphocytes,

and 12 per cent monocytes. Tibial bone marrow aspiration revealed a moderately cellular specimen with noticeable decrease in erythroid elements (erythroid/myeloid ratio = 1/4). No megaloblastic changes were seen. Flocculation tests of liver function, and values for bilirubin, cholesterol, protein-bound iodine, and electrolytes in the serum were within the normal range. Examination of the stools showed no trypsin. High electrolyte levels in the sweat (chloride 95 mEq., potassium 9.2 mEq., and sodium 103 mEq. per liter) and absence of tryptic and lipolytic activity in a viscid duodenal fluid established the diagnosis of cystic fibrosis of the pancreas.

Course. The patient was placed on an evaporated milk formula containing 3.7 Gm. per cent of protein, 4.5 Gm. per cent of fat, and 5 Gm. per cent of carbohydrate with adequate amounts of thiamine, riboflavin, nicotinic acid, and vitamin D, and took an average of 25 ounces a day. This regimen resulted in remarkable clinical improvement with a steady decrease in edema which subsided completely within a week. A rise in serum protein paralleled this. Concomitantly, reticulocytosis to a peak of 10.5 per cent occurred without administration of medicinal iron and was accompanied by an increase in hemoglobin concentration. After a continuous weight loss of 1 pound, 4 ounces, over an 8 day period, the patient began to gain weight. On the tenth day of hospitalization the patient was started on pancreatic enzymes (Viokase). He was discharged from the hospital a week later. The patient is under our care and is progressing satisfactorily.

Table I. Total proteins and values obtained by paper electrophoresis on serial samples of serum*

Patient and date	*Total protein*	*Albumin*		*α_1-globulins*		*α_2-globulins*		*β-globulins*		*γ-globulins*	
	Gm. %	*%*	*Gm. %*	*%*	*Gm. %*	*%*	*Gm. %*	*%*	*Gm. %*	*%*	*Gm. %*
Oberman et al.,[18] 1956 Mean values of healthy infants from 3 to 4 months of age	6.31	59.61	3.76	4.27	0.27	12.81	0.81	15.29	0.97	8.02	0.50
Green et al.,[19] 1960 Mean values of patients with cystic fibrosis from 3 to 12 months of age	6.03	54.08	3.30	5.17	0.31	14.92	0.88	13.42	0.79	12.42	0.75
Patient 1											
3/17/58	3.2	48	1.44	11	0.33	9	0.27	18	0.54	14	0.42
3/27/58	3.88	39	1.56	9	0.36	17	0.68	12	0.48	24	0.76
4/28/58	6.2	66	3.96	5	0.30	10	0.60	17	0.82	9	0.54
Patient 2											
9/17/58	3.95	41	1.64	8	0.32	20	0.80	14	0.56	16	0.64
9/25/58	4.8	48	2.40	7	0.35	15	0.75	19	0.95	11	0.55

*The results of protein electrophoresis on Patients 1 and 2 on admission and on subsequent occasions. Values are expressed as the per cent of total serum protein and absolute amount of each fraction per 100 ml.

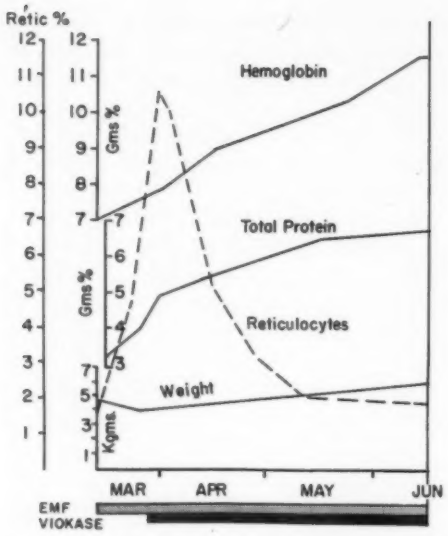

Fig. 2. Values for hemoglobin, total serum protein, reticulocytes, and body weight of Patient 1 on admission and over a period of 3 months. (*EMF*, Evaporated Milk Formula.)

Patient 2. A 3-month-old boy was admitted to the Children's Hospital Medical Center on Sept. 15, 1958, because of edema of both lower extremities. The patient was born normally at an estimated 38 weeks' gestation and weighed 5 pounds, 6 ounces. He was started on feedings of an evaporated milk formula. Because of an inadequate gain in weight and the appearance of an erythematous eruption at the age of 1½ months, the diagnosis of "milk allergy" was considered, and evaporated milk was replaced by a soya bean protein formula (Sobee). Except for one episode of upper respiratory infection the patient had no other symptoms until the day prior to admission when both legs were noted to be swollen.

Physical examination on admission revealed a moderately pale, well-developed infant. He weighed 9 pounds, 4 ounces. The temperature was 98° F., and the pulse rate 140 per minute. Examination of the head and chest showed no abnormality. The abdomen was soft and slightly distended. The spleen and liver were not enlarged. Marked pitting edema was present in the lower extremities, the external genitals, and the lower abdominal wall.

Laboratory data. The urine was found to be normal on repeated examinations. The hemoglobin concentration was 7.7 Gm. per cent. On the stained film of the peripheral blood there were normochromic, normocytic erythrocytes, and an adequate number of platelets. There were 10,500 white cells per cubic millimeter, of which 52 per cent were polymorphonuclear leukocytes, 2 per cent eosinophils, 5 per cent monocytes, and 41 per cent lymphocytes. The reticulocyte count was 2 per cent. Total serum proteins were 3.95 Gm. per cent, with an albumin fraction of 1.64 Gm. per cent. Results of liver function tests, serum bilirubin, and serum cholesterol were within normal range. A sweat test (chloride 102 mEq., potassium 16.5 mEq., and sodium 92 mEq. per liter) and duodenal aspiration yielded results consistent with the diagnosis of cystic fibrosis of the pancreas.

Course. On the day after admission the soya bean formula was discontinued, and the patient was given a similar evaporated milk formula and took an average of 24 oz. daily. On the fourth hospital day he was started on

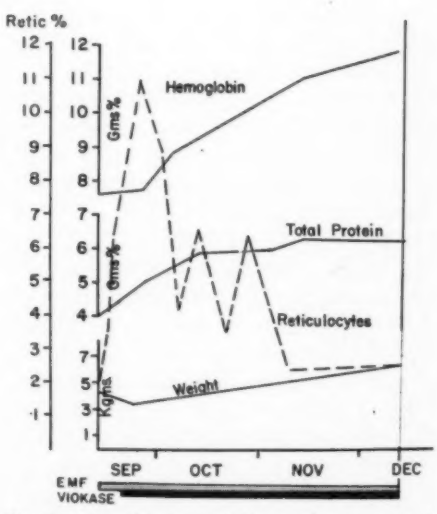

Fig. 3. Values for hemoglobin, total serum protein, reticulocytes, and body weight on admission of Patient 2 and over a period of 3 months. (*EMF*, Evaporated Milk Formula.)

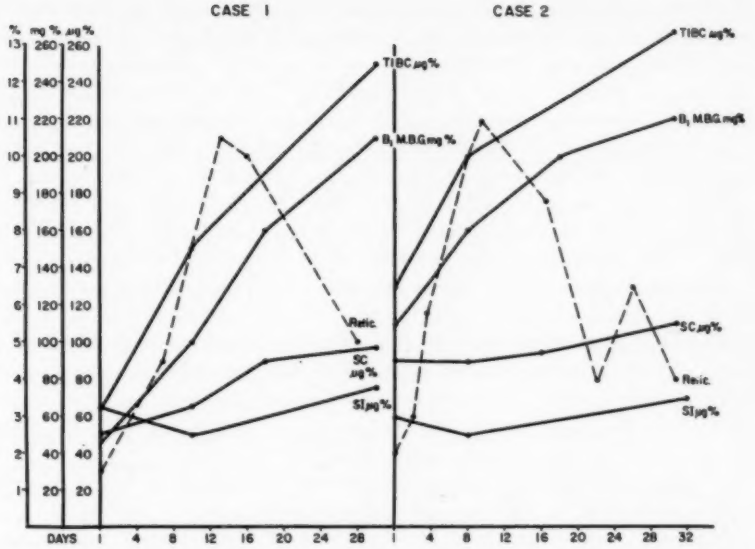

Fig. 4. Values for serum iron *(SI)*, total iron-binding capacity *(TIBC)*, β_1-metal-binding-globulin, serum copper *(SC)*, and reticulocytes on admission and during the recovery phase.

pancreatic substitution therapy (Viokase). Concomitant with a rise in total serum proteins, the patient improved, and the edema subsided within 6 days. The reticulocyte level rose to 11 per cent without addition of medicinal iron to the diet and there was a steady rise in hemoglobin concentration. After a loss of 1 pound, 4 ounces in weight, in the first 7 hospital days, the patient began to gain and was discharged on the eleventh day. He is seen regularly in our Nutrition Clinic and his progress remains satisfactory.

RESULTS OF SPECIAL STUDIES

Figs. 2 and 3 show the values for hemoglobin, total serum protein, reticulocytes, and body weight on admission and over a period of 3 months thereafter. In each patient, following the institution of an adequate evaporated milk formula, total serum protein and reticulocyte levels rose, followed by a gradual increase in the hemoglobin concentration.

The results of electrophoretic analysis of serum proteins for each patient on admission and subsequently during the recovery phase are shown in Table I and are compared to values obtained by Oberman and others[18] in healthy infants 3 to 4 months old and, also, to those determined by Green and co-workers[19] in patients with cystic fibrosis from 3 to 12 months of age. In Patient 1 all fractions, except α_1-globulins, were reduced. In patient 2, the albumin and β-globulin fractions were significantly decreased.

Values for serum iron, total iron-binding capacity (TIBC) and its percentage of saturation for each patient on admission are shown in Table II and compared to mean normal value for this age group obtained in our laboratory. Hypoferremia was present in both patients and was associated with the total lack of unsaturated iron-binding capacity (UIBC) in serum from the first patient, and with unusually low values in the second. The absence of UIBC has also been reported by Lahey and associates[6] in some patients with kwashiorkor, and by Riegel and Thomas[20] in an 85-year-old woman with chronic anemia. The concentration of serum iron and TIBC measured on different occasions during a 1 month period are shown in Fig. 4.

Table II. Biochemical values of Patients 1 and 2 obtained within 2 days following admission compared with the mean values of healthy infants

Patient	*Serum iron (μg/100 ml.)*	*Total iron-binding capacity (μg/100 ml.)*	*Saturation %*	*β_1-metal-binding globulin (mg./100 ml.)*	*Serum copper (μg/100 ml.)*
Healthy infants 2 to 5 months	95 ± 25	280 ± 30	33	230 ± 27	98 ± 15
Patient 1	65	65	100	45	50
Patient 2	60	130	46	110	90

Table III. Total serum protein and values obtained by paper electrophoresis on serial samples of serum in a newborn infant with cystic fibrosis placed on Sobee

	Total protein	*Albumin*		*α_1-globulins*		*α_2-globulins*		*β-globulins*		*γ-globulins*	
Date	*Gm. %*	*%*	*Gm. %*	*%*	*Gm. %*	*%*	*Gm. %*	*%*	*Gm. %*	*%*	*Gm. %*
10/26/60	6.8	60	4.08	6	0.41	10	0.68	8	0.54	16	1.09
10/31/60	5.1	65	3.30	3	0.15	5	0.25	7	0.36	20	1.04
11/ 6/60	4.8	66	3.17	2	0.10	9	0.43	4	0.19	19	0.91
11/27/60	4.1	61	2.56	3	0.13	9	0.38	12	0.50	15	0.63

In comparing the quantitative alteration of total serum iron-binding capacity to that of the β-fraction of serum globulins during the recovery phase, no correlation could be demonstrated. This is perhaps not surprising since the metal-binding globulin is only one of the proteins that migrates with the β-globulin. In order to confirm the results of iron-binding capacity studies and also to ascertain whether or not, in the absence of UIBC, the circulating iron was actually bound to β_1-metal-binding globulin, immunochemical measurements of metal-binding globulin were performed. The results are shown in Table II and compared to mean values obtained in 31 healthy infants from 2 to 5 months of age. It is apparent that the β_1-metal-binding globulin was present in greatly reduced amounts; its concentration in the serum of both patients rose in parallel with the increase in TIBC (Fig. 4).

The concentration of serum copper in each patient on admission is shown in Table II, as is the mean value obtained in 27 healthy infants from 2 to 5 months of age. Significant hypocupremia was present in only the first patient. Subsequent quantitative alterations of serum copper concentrations for each patient are shown in Fig. 4.

The half-life of I^{131}-labeled albumin in the first and second patients was 12.5 and 12 days, respectively, which falls within the range of values found in this laboratory in normal children and by Sterling[17] in normal adults.

DISCUSSION

Hypoproteinemia. The normal half-life of labeled albumin makes the possibility of hypercatabolic hypoproteinemia syndromes, such as transient dysproteinemia[21] or exudative enteropathy,[22] highly unlikely. Absence of measurable protein in the urine of these patients excludes the possibility of the nephrotic syndrome. Severe liver damage in children[23, 24] could interfere with protein synthesis and consequently lead to hypoproteinemia. However, in these infants, no signigficant disturbance of hepatic function could be detected by conventional tests. In addition, as seen in Figs. 2 and 3, the hypoproteinemia was corrected readily by feeding an evaporated milk formula and by administration of pancreatic enzymes. Therefore, the

most probable cause for the hypoproteinemia in these infants would seem to have been faulty utilization of dietary protein. The pancreatic deficiency alone, however, does not seem to have been solely responsible for the protein failure, for the plasma proteins began to rise while the patients were fed only an evaporated milk formula, before pancreatic enzymes were added to the diet. Both patients developed edema while being fed a soya bean protein preparation and improved markedly when an evaporated milk formula was reinstituted. Similar observations in children with cystic fibrosis who were fed soya bean protein have been made by Fleisher and associates.[25] The adequacy of soya bean protein in promoting nitrogen retention in normal infants has been emphasized by Fomon and May.[26, 27] However, DeMaeyer and Vanderborght,[28] in studying nitrogen retention in children with kwashiorkor, on diets containing either animal or vegetable protein noticed that cow's milk was superior to soya bean flour as a source of protein. These authors also found that their patients, like most patients with kwashiorkor, presented signs of pancreatic deficiency. Fleisher and co-workers[29] found that an infant with cystic fibrosis of the pancreas and hypoproteinemia excreted as much as 80 per cent of the ingested nitrogen as fecal nitrogen while receiving the soya bean formula. The addition of Viokase to the formula resulted in a significant decrease in the fecal nitrogen. These observations, in conjunction with our own, suggest that, in the presence of pancreatic insufficiency, the digestion or absorption, or both, of protein from the soya bean is more affected than that from cow's milk. In order to confirm further the faulty utilization of soya bean protein in the presence of pancreatic deficiency, a 4-day-old infant with cystic fibrosis was placed on soya bean formula (Sobee) of which he took an average of 20 oz. daily. Following this regimen the total serum protein dropped from 6.8 to 4.2 Gm. per cent over a period of 25 days. Table III shows four selected values.

Anemia. The age at onset of anemia, the absence of hypochromic erythrocytes on stained smears of peripheral blood, and decreased erythroid activity in the bone marrow of these patients make the possibility of iron depletion highly unlikely, especially since the anemia was corrected without a transfusion of blood or the addition of medicinal iron to the diet. The absence of megaloblastic changes in the bone marrow performed intially excludes the possibility of folic acid or vitamin B_{12} deficiency. The possibility of an exaggeration of physiologic anemia coinciding with hypoproteinemia is unlikely for in four other infants with cystic fibrosis, edema, and hypoproteinemia studied by Fleisher,[30] the hemoglobin concentration ranged from 9.2 to 6.4 Gm. per cent. Reticulocytosis with subsequent rise in hemoglobin concentration was seen in each patient and followed the feeding of evaporated milk formula and pancreatic enzymes and accompanied the rise of plasma protein. These findings suggest that protein deficiency was probably responsible for the low hemoglobin concentrations.

Decreased hemoglobin synthesis as the result of inadequate protein intake has been demonstrated in several animal species. Weech and others[4] found that in dogs which were fed a low protein diet the hemoglobin dropped to 80 per cent of the initial value. Cartwright and Wintrobe[1] showed that low or absent protein from the diet of growing swine resulted in hypoproteinemia and a normochromic, normocytic anemia of moderate degree. Orton and Orton[3] produced mild anemia in weanling rats fed on a protein-deficient diet supplemented only by vitamins. Pearson and co-workers[5] observed that in iron-deficient rats the rise of hemoglobin concentration after institution of iron was significantly lower in protein-deficient animals, compared with those having an adequate protein intake. Hallgren[2] found that the deleterious effect of protein deficiency upon hemoglobin synthesis was not related to the amount of iron stored.

In the presence of low protein intake some competition must exist among various demands for protein synthesis. In a series of experiments performed by Robscheit-Rob-

bins and associates,[31] dogs were rendered hypoproteinemic and anemic by bleeding and low protein diet. When protein was supplied in various forms, it was found that in all circumstances hemoglobin synthesis had high priority over plasma protein formation in that for every gram of plasma protein, 2 to 4 Gm. of hemoglobin were added. Since, in the normal dog's circulation the concentration of hemoglobin (18 to 20 Gm. per cent) is about 3 times that of plasma proteins, these authors postulated that the priority of hemoglobin over plasma protein synthesis was most likely the result of this physiologic ratio. Figs. 2 and 3 indicate that in our infants, during the phase of recovery, the rise in concentration of hemoglobin paralleled that of the plasma proteins and showed no such priority. This discrepancy may be partially explained by a lower hemoglobin/plasma protein ratio (12/6) in the blood of human beings at this age. However, it has been shown[2] in protein-deficient rats that when there is an increased demand for protein formation, as in pregnancy or lactation, hemoglobin production is more affected than the synthesis of other body proteins. These findings suggest that in infants the rapid rate of growth with its heightened protein requirements may be a second factor placing hemoglobin formation in a less favorable position with respect to plasma protein synthesis.

Hypoferremia and hypocupremia. The idea that hypoferremia in our patients was the result of iron depletion without demonstrable blood loss is unlikely. The low serum iron values found in these infants presumably reflected a defect in the transport mechanism as shown by unusually low values of β_1-metal-binding globulin. It has been shown[32] that, in rats, both acute and chronic protein depletion results in a disturbance of iron metabolism with reduced incorporation of radioiron into hemoglobin and increased iron deposits in the liver. The eventual rise in serum iron without administration of medicinal iron or transfusions of blood also favors the assumption of inadequate mobilization and transport rather than depletion of iron stores. Fig. 4 indicates that, despite a rapid increase in the concentration of β_1-metal-binding globulin, the concentration of iron in the serum dropped further. However, in each patient this accentuation of hypoferremia accompanied the reticulocytosis, and was most likely the result of increased iron utilization during active erythropoiesis. Similar observations have been made by Cartwright and Wintrobe[1] in hypoproteinemic swine treated with casein.

The hypocupremia present in the first patient could also be assumed to be due to a defect in the transport mechanism. It has been demonstrated that most of the copper in serum is tightly bound to an α_2-globulin, ceruloplasmin, although a small amount is loosely bound to other proteins.[33] Subsequent studies have shown a high degree of correlation between quantitative alterations of the α_2-globulin fraction and the concentration of copper in the serum of human subjects.[34] The present data are consistent with these correlations. However, in patients with kwashiorkor, with low values of serum copper, the absolute amounts of the α_1- and α_2-fractions of the serum globulins were essentially unaltered.[6] These findings suggest that the mechanism of hypocupremia in hypoproteinemic states needs further study.

SUMMARY

Anemia, with diminished concentration of serum iron and total iron-binding capacity as the result of protein failure, has been described in two young male infants suffering from cystic fibrosis, who had been fed formulas based on soya bean protein. Hypocupremia was also present in one of the patients. In the absence of detectable protein loss or increased protein degradation the hypoproteinemia appeared to be the result of inadequate utilization of this vegetable protein by these infants with defective protein digestion. The age at onset of anemia, the normochromic, normocytic appearance of the erythrocytes, and the decreased erythroid activity in the bone marrow all made the possibility of iron deficiency unlikely. Rapid and sustained reticulocytosis following

an adequate intake of high-quality protein without additional iron suggested that protein deficiency was responsible for the low hemoglobin concentrations. In this connection the role of inadequate protein intake in the quantitative disturbance of hemoglobin synthesis is discussed. While the hypoferremia is thought to reflect a defect in the transport process, the mechanism whereby protein deficiency leads to hypocupremia remains less certain.

We wish to thank Dr. David Gitlin for his advice and the supply of anti-β_1-metal-binding-globulin rabbit serum, Dr. Fernando Monckberg for the serum copper determination, and Dr. Morris N. Green for the electrophoretic studies of protein reported in this paper.

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Familial infantile cortical hyperostosis

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INFANTILE cortical hyperostosis (I.C.H.) no longer presents a diagnostic problem, but its etiology is still obscure. In a full discussion of the nature of the disease Sidbury and Sidbury[1] considered that there was insufficient evidence to indicate that infection, trauma, allergy, endocrinopathy, maternal causes, birth factors, or dietary aberrations caused the disorder; they supported the suggestion made by Barba and Freriks[2] that it was vascular in origin and suggested that it was due to an inherited defect in the arterioles supplying the affected areas. Caffey,[3] reviewing this syndrome to which his name is now firmly attached and which he had originally described, discarded all etiological suggestions and speculated that only a study of the mothers of involved infants would eventually unravel the baffling problem of the cause and pathogenesis of the disease. An overriding reason for Caffey's statement was the knowledge that the disease develops either in utero or soon after delivery, so that intrauterine factors appear to condition its development in some instances, if not in all. Sidbury and Sidbury[1] emphasized genetic factors because the disease sometimes has a familial incidence; 2 siblings had had the disease in the group of families which they reported. Van Zeben[4] reported the disease in 2 siblings and a cousin; Kitchin[5] found the condition in a child whose mother had probably had the disease in infancy. Idbohrn, Lindegard, and Lindquist[6] reported the disease in second cousins in a Scandinavian family. Boyes and Demy[7] observed the disease in 2 siblings and inferred from the family history that the mother of their patient, her sister, her mother, and her maternal grandfather also had the disease; if this were so, the disease had been manifest in 4 successive generations. More recently Tampas and colleagues[8] examined a family in which the disease was present in 11 members in 2 generations; 6 of the cases occurred in 4 families whose parents had all been siblings, the remaining 5 cases occurred in 2 families in the following generation. The father of 4 of these children had also had infantile cortical hyperostosis in infancy. We are recording a further example of the familial incidence of the disease, thus lending further support to the suggestion that genetic factors play a part in its causation.

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***Markle Scholar in the Medical Sciences.*

CASE HISTORY

In February, 1960, a child with suspected rickets was referred to the University Hos-

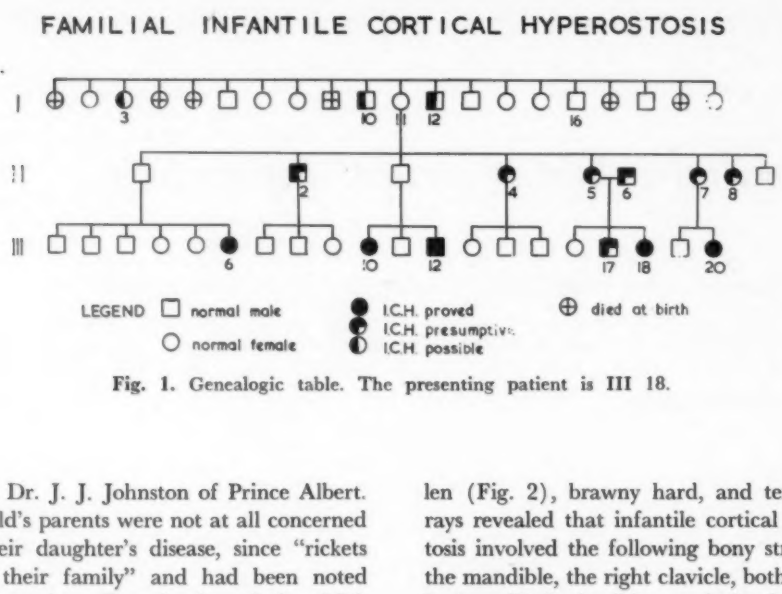

Fig. 1. Genealogic table. The presenting patient is III 18.

pital by Dr. J. J. Johnston of Prince Albert. The child's parents were not at all concerned with their daughter's disease, since "rickets ran in their family" and had been noted in 3 generations. Examination of the child, however, revealed that she had infantile cortical hyperostosis, and a careful history suggested that it was this disorder which had been present in the 3 generations (Fig. 1). After the child had been examined and studied, her parents were interviewed and x-rayed, and so was their second child, who had a presumptive history of infantile cortical hyperostosis in infancy; all 3 were found to be radiologically normal. Subsequently, the child's grandmother, I 11, was interviewed, and letters were dispatched to all her living siblings; replies were received from the majority.

The relevant findings, starting with the propositus, III 18, were as follows.

Generation III. The presenting patient, III 18, had a normal antenatal and perinatal history. At the age of 5 weeks the legs, more particularly the right, became swollen and tender between the knee and the ankle; the following week the face became chubby and the lower jaw appeared to fill out. Six weeks later the forearms became swollen and tender. With the onset of these swellings she became irritable but not obviously febrile. On examination at the age of 4 months the significant findings were in the jaw, the forearms, and the lower limbs; these were swollen (Fig. 2), brawny hard, and tender. X-rays revealed that infantile cortical hyperostosis involved the following bony structures: the mandible, the right clavicle, both humeri, both radii, both tibias, and the left fibula (Figs. 3, 4, and 5). She had a leukocytosis, but the erythrocyte sedimentation rate was normal. She gradually made a complete recovery, but at the age of 15 months she appeared to have an exacerbation and the left forearm and the distal parts of both lower limbs again became swollen. At the age of 16 months, hyperostosis of the left tibia was still present, and there was residual bowing of the right tibia.

A cousin, III 6, was seen at the age of 3 months because the legs had become swollen and tender at 2 weeks of age. At the age of 4 weeks the right forearm also became swollen, and she developed a pseudoparalysis of it. By the time she was 3 months of age her cheeks had also become swollen. Radiologic studies at this time revealed infantile cortical hyperostosis involving both mandibles, the right scapula, the right radius, both tibias, and several ribs.

Another cousin, III 10, was seen at the age of 7 months with a firm tender mass on the medial aspect of the right tibia. Radiologic examination revealed marked periosteal new bone formation, and although no bony injury was seen, it was thought that she had a traumatic periostitis. Seven months later it had resolved. In retrospect, it seems cer-

tain that this child had infantile cortical hyperostosis.

III 12, a sibling of III 10, was seen at the age of 4 months because he had developed firm, tender swellings on the medial aspects of both tibias. Radiologic examination revealed infantile cortical hyperostosis involving both mandibles and both tibias; there was no clavicular involvement.

A sibling of the propositus, III 17, developed obvious swelling of the mandibles and became irritable when he was 2 to 3 months of age; the swelling persisted for a month and then gradually subsided. No radiologic studies were carried out on this child but the appearance of his face was similar to that of his sister, III 18.

A cousin, III 20, was taken home from the hospital at the age of 6 days and his mother noticed immediately that his legs were swollen above and below the knee. Radiologic examination at the age of 2½ months revealed hyperostosis of both mandibles, the femora, the humeri, and the fifth and sixth right ribs.

Generation II. II 2 developed swollen painful gums and a fever when he was 4 to 5 months of age. He may or may not have had infantile cortical hyperostosis; the story is not sufficiently characteristic to be diagnostic.

II 4 developed brawny swellings of both arms and legs and the lower jaw, associated with fever and irritability, when she was 1 month of age. She had recovered completely by the age of 12 months. She is now normal and her legs are straight.

II 5 developed swellings of the legs and, to a less extent, of the arms and cheeks when she was 4 weeks old; at this time she also became irritable. She had fully recovered by the age of 15 months. She is now normal and her legs are straight.

II 6, the father of our first patient III 18, was the second of twins. At 6 weeks of age, when his normal twin was beginning to smile, his mother became concerned because he became irritable and cranky; at this time his clavicles and the back of his head became swollen and tender. This disorder was ascribed to "rickets." He had fully recovered by the time he was 11 months old; he is now normal in all respects.

II 7 developed swollen arms and to a less degree swollen cheeks when she was 6 weeks old. She had fully recovered by the age of 9 months. She is now normal and has no bowing of the legs.

II 8 developed a very conspicuous swelling

Fig. 2. Child (III 18) aged 4 months with infantile cortical hyperostosis.

Fig. 3. Roentgenogram of skull and clavicles of Patient III 8 showing involvement of mandible and right clavicle.

of the cheeks, arms, and legs at the age of 4 to 5 weeks. Her symptoms had subsided by the age of 6 months. She is now clinically normal and has no bowing of the legs.

Generation I. We did not intend initially to include generation I in this report but are doing so because of the close similarity this family bears to the one described by Boyes and Demy.[7] We have, in addition, seen infantile cortical hyperostosis involving a tibia followed by bowing in a member of the third generation of this family (III 18) leading us to conclude that bowing may be a sequel of the disease. The relevant facts, as we have obtained them, are as follows:

I 3 had swollen legs during the first year of life and is now bowlegged.

I 10 had swollen legs and arms during the first year of life and is now a little bowlegged.

I 12 had his legs placed in casts in infancy; they are now normal.

I 16 had misshapen feet at birth; they were put in casts when he was 2 weeks of age. He is now normal.

DISCUSSION

The first patient in this family to be seen by us, III 18, undoubtedly had infantile cortical hyperostosis. The diagnosis in III 6, III 12, and III 20 has also been established. It is almost certain that III 10 and III 17 also had the disease. There are, therefore, 6 children, the most at risk being 20, in four related families, who have had infantile cortical hyperostosis. With regard to the previous generation, there can be little doubt that II 4, II 5, II 7, and II 8 had the disease. The history in each instance is characteristic of the disease, and the grandmother, I 11, asserts that the disease which these children had was the same as that manifested by III 17 and III 18. It would seem probable that II 6, the father of III 18, also had it.

The nature of the disease in I 3, I 10, and I 12 is uncertain, but all 3 may have had infantile cortical hyperostosis. The family his-

Table I. Key to family tree

I	3	Bernadette S.	
	6	Roland A.	
	7	Beatrice A.	
	8	Jean B.	
	10	Leopold A.	
	11	Clare L.	
	12	Conrad A.	
	13	Ferdinand A.	
	14	Adrianne S.	
	15	Theodora A.	
	16	Roger A.	
	18	Germaine A.	
	20	Marie Martha A.	
II	1	Fernand L.	Saskatchewan
	2	Maurice L.	British Columbia
	3	Emile L.	British Columbia
	4	Beatrice B.	Saskatchewan
	5	Adrienne LeB.	Saskatchewan
	6	M. LeB.	Saskatchewan
	7	Jeanine W.	Alberta
	8	Bernadette L.	Saskatchewan
	9	Raymond L.	Saskatchewan
III	6	Yvette L.	
	10	Judy L.	
	12	Dale L.	
	17	Maurice LeB.	
	18	Claudette LeB.	
	20	Anita W.	

tory resembles closely that of the family reported by Boyes and Demy,[7] in which the disorder may have been present in four generations. Bowing of the legs is certainly not common, but it may occur as described in the 3 patients of Van Zeben,[4] and in our patient III 18. In the atypical case reported by Kitchin,[5] infantile cortical hyperostosis was also followed by bowing; the mother of this child also had a disorder simulating the disease in infancy.

Familial disorders may be environmental in origin, but, as Caffey[3] has indicated, if it is so in this disease, it is the intrauterine environment which is at fault. Alternatively, the disorder may be genetically determined, as Sidbury and Sidbury[1] first suggested. If this is so, its appearance in two and probably in three generations in the family described here would suggest that it is carried by a dominant autosomal gene, with reduced penetrance or expressivity. This mode of transmission would appear to apply also to the families described by Kitchin[5] and by Boyes and Demy.[7] The mode of inheritance in the family described by Tampas and associates[8] is almost certainly on the same basis; dominant transmission with reduced penetrance would fit their data more satisfactorily than recessive transmission. In most families, however, where there has been a familial incidence of I.C.H., the parents have been unaffected; only their children have had the disease. In these families a recessive mode of inheritance would be the more probable. One of us (G. H. H.) has recently reviewed all cases with I.C.H. seen at the Harriet Lane Home between the years 1944 and 1957. The involved children came from 33 families. In only one family were siblings affected; in this family 2 of 5 children had I.C.H. In 2 other instances first cousins had the disorder. More recently an additional local Saskatoon family has been encountered in which the disorder developed in 2 of 3 siblings; the parents had not had the disease. With the exception of the families in which first cousins were affected, a dominant mode of transmission would appear to be unlikely.

Fig. 4. Roentgenogram of forearms of patient III 8 showing involvement of both radii.

Fig. 5. Roentgenogram of lower limbs of patient III 8 showing involvement of both tibias and left fibula.

Most cases of I.C.H. appear to be sporadic, but some are undoubtedly familial; in the latter instances its development is probably conditioned either by a recessive or less commonly by a dominant autosomal gene. The severity and extent of the disease is extremely variable, and it would seem unlikely that a single factor conditions its development.

SUMMARY

A family is described in which infantile cortical hyperostosis developed in two and possibly in three generations. This observation lends support to the suggestion that, in some instances at least, this disorder is a familial disease. In the family here recorded it would appear to be transmitted by a dominant autosomal gene with reduced penetrance.

ADDENDUM

Case II 4, B. B., has just given birth to her fourth child. The latter is now 3 weeks old and has infantile cortical hyperostosis involving the left mandible, right clavicle, both radii, left femur, and both tibiae. This disease is, therefore, present in the third generation in 5 of the 6 families at risk, lending additional support to the dominant character of the disease in this family.

Our thanks are due to Dr. J. J. Johnston who asked us to see the child here reported upon, to Dr. A. E. Buckwold who gave us access to a second family in which siblings had the disease, and to Professor T. J. Arnason for his help and advice.

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Morquio-Ullrich's disease

Report of 2 cases

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EVIDENCE is presented in this report that Morquio-Ullich's disease is a mucopolysaccharidosis.

In 1917, Hunter,[1] for the first time, described a "rare disease," which subsequently became well known under different synonyms, Hunter-Hurler's disease, Hurler's disease, von Pfaundler-Hurler's disease,[2] gargoylism,[3] lipochondrodystrophy, and dysostosis multiplex. Several reviews of this condition have appeared recently.[4-6] The first descriptions of another skeletal disease appeared in 1929 by Morquio[7] and Brailsford.[8] This condition became known as Morquio's disease, chondroosteodystrophy, Brailsford-Morquio's disease, osteochondrodystrophia deformans[9] and has been reviewed recently by Grebe.[10] These conditions were originally considered as 2 separate disease entities. Later investigators[11] postulated a close relationship between the 2 diseases on the basis of cases displaying manifestations of each condition. Some of these cases belong in a third category, called Morquio-Ullrich's disease,[12] which was separated from Hurler's disease and Morquio's disease in 1954. Two cases of Morquio-Ullrich's disease will be discussed in this paper.

Gargoylism or Hurler's disease in its typical form is easily distinguished from Morquio's disease by the presence of a number of extraskeletal changes such as corneal opacities, deafness, hepatomegaly, splenomegaly, progressive mental deterioration, neurological disturbances as pareses, cardiovascular changes, alterations of the connective tissue as evidenced by thickened skin, gargoyle face, limitation of joint movements, and hernias. One or several of these symptoms are lacking in the majority of cases, however,[13] particularly in younger patients since some of the manifestations develop only after a number of years. Furthermore, a milder form of Hurler's disease, without corneal opacities and with little or no mental retardation, with longer life expectancy, though with a higher tendency to deafness and cardiac involvement, and with a recessive sex-linked mode of inheritance, has been

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described in recent years. Mental retardation, on the other hand, occurs also in Morquio's disease and even with a somewhat higher incidence than in the average population. Hepatosplenomegaly has been described in exceptional cases of Morquio's disease.[6]

The radiologic picture of the osseous changes of Hurler's disease and Morquio's disease are identical according to one group of authors,[10, 11, 17] whereas others[12, 14, 15] have no difficulties in classifying a case on the basis of the skeletal changes alone; in particular, the hook-shaped lumbar vertebrae of Hurler's disease are distinctly different from the platyspondyly of Morquio's disease. The elongated sella turcica and the poorly tubulated long bones in Hurler's disease are other, but less constant, differential signs.

Hurler's disease and Morquio's disease can be differentiated by hematologic, biochemical, and histologic methods as well. In about 50 per cent of the cases of Hurler's disease, the so-called Reilly granulations are found in the white cells of the peripheral blood.[16] In some cases they are found in the lymphocytes, in other cases in the granulocytes.[18, 19] We were able to find Reilly granulations in almost all of our patients with Hurler's disease. The Reilly granules disappear if hyaluronidase is added to the slides.[20] Vacuolated cells with peculiar purple inclusions are found (Buhot cells) in the bone marrow in some cases of Hurler's disease.[21-22] Increased amounts of mucopolysaccharides, in particular chondroitin sulfate B and heparitin sulfate, have been found in the urine of most patients with Hurler's disease[23-24] but not in heterozygous gene carriers.[27] Simplified screening tests have been described.[25-26] Increased amounts of mucopolysaccharides were also found in liver, spleen, and brain.[28-30]

The histologic findings in these 2 disease entities have not been compared in a critical way, but the lesions are considered to be unlike in most respects. Several rather extensive studies of Hurler's disease, including the reports of Lindsay[31] and Dawson,[32] have given a fairly consistent account of the histologic abnormalities found. These observations have been instrumental in establishing the concept of Hurler's disease as a disorder of the mucopolysaccharide metabolism, with the accumulation of the products of abnormal metabolism in many organ systems.

The necropsy report of Einhorn, Moore, and Rowntree[33] and the biopsy studies of Shelling[34] are among the few published accounts of the histologic abnormalities of Morquio's disease. No characteristic extra-

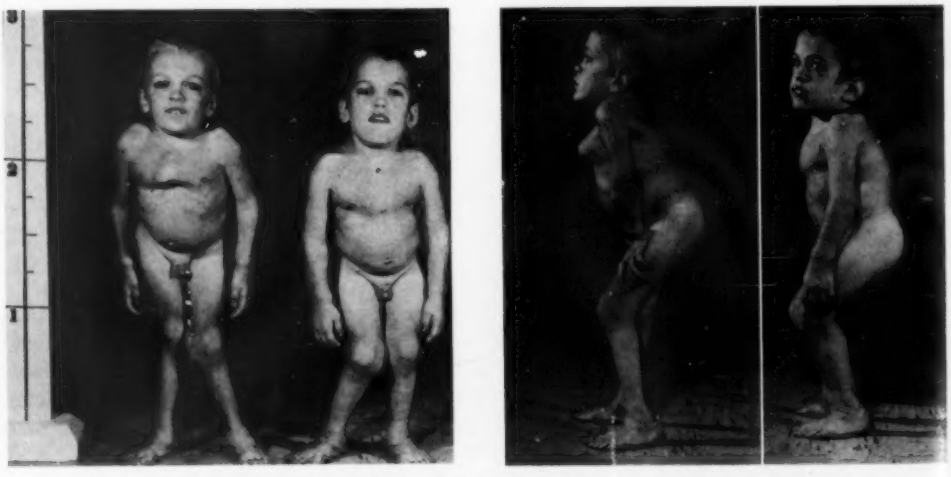

Fig. 1. Two brothers with Morquio-Ullrich's disease. Case 1, left. Case 2, right.

skeletal lesions have been noted, and the skeletal changes have not been considered to be unique or pathognomonic histologically. The findings have been interpreted as involving irregular growth of articular cartilage and epiphyses with faulty enchondral bone formation and degeneration and focal aseptic necrosis of bone and cartilage. These abnormalities have been compared to the more uniform and symmetrical skeletal growth disturbances of achondroplasia. Evidence of abnormal mucopolysaccharide storage has been notably absent and has been the most conspicuous negative histologic feature of Morquio's disease to differentiate it from Hurler's disease.

Recent research indicates that a disorder of the metabolism of the mucopolysaccharides is present in Hurler's disease which is responsible for the skeletal and the extraskeletal symptoms of this disease. A defect of one or more enzymes involved in the metabolism of the mucopolysaccharides has been postulated. In Morquio's disease a metabolic disorder has not been found. Hurler's disease and Morquio's disease could, therefore, be considered as 2 entirely different etiopathogenic entities. In the light of this concept, it is surprising to find 17 cases reported in the recent literature in which a Morquio's disease—like external appearance and radiologic changes are associated with typical Hurler's disease changes. Hepatomegaly was found in 2 cases,[12] corneal opacities in 17 cases, [12, 17, 35-38] deafness in 3 cases,[12, 37] and Reilly granulations in 2 cases.[17] Ullrich classified such cases as late forms of Hurler's disease,[39] yet this led to confusion with cases of true Hurler's disease with late-appearing symptoms. Wiedemann[12] suggested, therefore, the name Morquio-Ullrich's disease, indicating that this probably represents a separate disease entity.

Two cases of Morquio-Ullrich's disease followed in this institution for several years will be presented here.

CASE REPORTS

Case 1. D. E. was born Aug. 21, 1949. The family history revealed that one

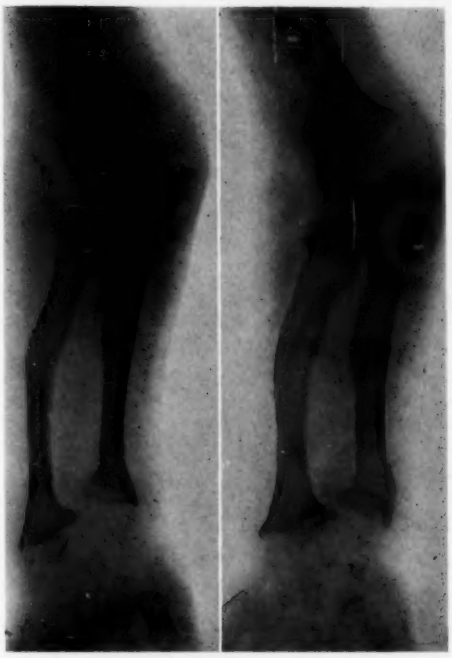

Fig. 2. Roentgenograms of the arms of Case 1, left, and Case 2, right.

Fig. 3. Roentgenograms of the hand of Case 2.

younger brother (Case 2) has a similar condition. The parents and another brother are in perfect health. The family is of English-Norwegian extraction.

The mother felt well during her pregnancies. The delivery of this child was normal. The birth weight was 3,570 grams. The early psychomotor development was normal. He sat at 6 months of age, crawled at 8 months, and walked without support at 18 months. He was toilet trained at 2 years. The first teeth appeared at 3 months, and the first dentition was completely erupted at 18 months. Between 6 and 9 years of age he had measles, mumps, chickenpox, and several attacks of pneumonia.

The parents noticed some flaring of the rib cage at birth. At the age of 5 months, the sternum began to protrude, at 1 year the first signs of kyphosis were observed. Further deformities developed progressively in the subsequent years. His appetite was good in the first year of life and decreased in the following year.

Fig. 4. Roentgenograms of the pelvis. Case 1, above. Case 2, below.

An examination performed at the age of 2½ years already revealed a growth retardation of 10 cm. per hypertrophy of the tonsils and the adenoids, considerable muscular hypotonia and hyperlaxity of the joints, a thoracolumbar kyphosis, severely protruded chest, knock-knees, and flatfeet. Blood chemical determinations which included carbon dioxide–combining power, nonprotein nitrogen, serum calcium, inorganic phosphate, and alkaline phosphatase were within normal range. A balance study* revealed no retention of calcium, a phosphorus retention of 11, and a nitrogen retention of 15 mg. per kilogram of body weight in 24 hours.

In the following years, dwarfism and deformities grew progressively worse. He began school at the age of 6 years, but he changed schools several times because he had difficulties with schoolwork and adjustment. At 10½ years, he and his brother entered the Hospital School for Handicapped Children in Iowa City. He adjusted better there though he was extremely apprehensive. He was overly conscious of his deformity and had not yet accepted his handicap. Two Wechsler Intelligence Tests revealed I.Q.'s of 91 and 93. He had considerable difficulty in walking, tired easily, and had to rest after walking short distances.

At 10½ years of age, body length was 91 cm., body weight was 16 kilogram, sitting height was 46 cm., armspan 90 cm., and the frontooccipital head circumference was 53 cm. The patient was a stunted disproportioned dwarf with a rather big head, but without gargoyle features (Fig. 1). The neck and trunk were short, the extremities relatively long. The anteroposterior diameter of the chest was increased in size. The sternum was bulging. There was considerable kyphosis of the lower thoracic spine. The lower end of the short chest was flaring. The boy stood and walked in a semicrouched position with semiflexed hips and knees. Elbows, wrists, knees, and ankles were widened. There was genu valgum and pes valgus bi-

*Performed by Dr. G. Stearns.

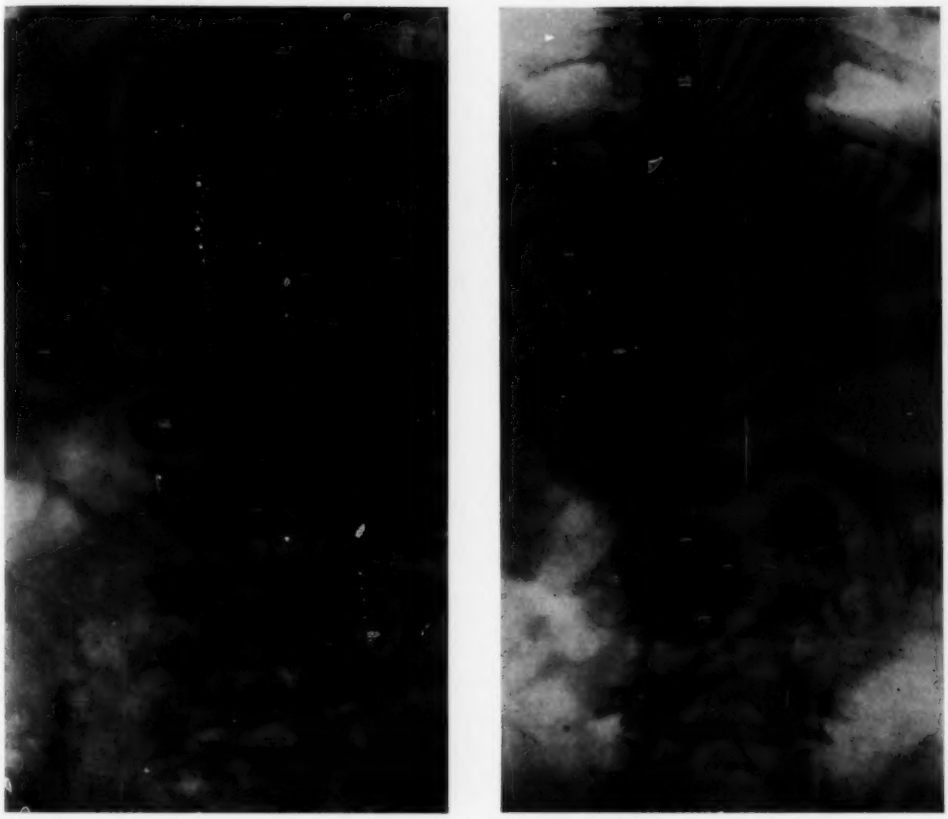

Fig. 5. Anteroposterior roentgenograms of the spine of Case 1, left, and Case 2, right

laterally. With the knees together, the distance between both medial malleoli was 12 cm. There was hyperlaxity of the joints and muscular hypotonia. The musculature was underdeveloped. The slit lamp examination revealed corneal opacities. A detailed report of the eye findings has been described elsewhere.[40] External ears and nose were normal. An audiometric examination revealed a hearing loss of 10 to 30 decibels in the frequencies between 125 and 4,000 cycles per second. The patient had 26 permanent teeth. Their enamel was considerably hypoplastic. The tips of the cuspids of the posterior teeth appeared sharp, thin, and piled up. The tonsils and adenoids were hypertrophic. No enlarged thyroid gland was felt. There was a systolic murmur in the left parasternal region of the third interspace. The lower margin of the liver was felt 2 cm. below the costal margin. No spleen was felt. The genitals were normally developed. No abnormal neurological signs were found.

Routine examination of the urine, including a ferric chloride test, was normal. A complete blood count was normal. Reilly granulations were found in 65 per cent of the polymorphonuclear neutrophilic leukocytes. Calcium, phosphorus, alkaline phosphatase, total cholesterol, and total lipids in the serum were within normal limits. A 24-hour urine specimen contained 1.1 mg. 17-hydroxysteroids (normal range for adults 1.1 to 1.7 mg.). A screening test for urinary

Fig. 6. Lateral roentgenograms of the spine of Case 1, left, and Case 2, right.

mucopolysaccharides with Alcian blue was positive.* The bone marrow again revealed Reilly granulations in the granulocytes; no Buhot cells were found. Electrocardiogram, electromyogram, and electroencephalogram were all normal.

Case 2. Dav. E. was born Nov. 16, 1950. This boy is the brother of Patient 1. Pregnancy and delivery had been normal; his birth weight was 3,270 grams. His early psychomotor development was normal. His deciduous teeth erupted very early as in Patient 1. He experienced repeated infections of the throat and ears until tonsils and adenoids were removed. An inguinal herniorrhaphy was performed at 2 years of age.

At 1½ years of age he was hospitalized in the Department of Orthopedics. The physical findings, at that time, were very much the same as those of Patient 1. A balance study revealed no retention of calcium and phosphorus and a nitrogen retention of 232 mg. per kilogram of body weight in 24 hours.

*We are indebted to Dr. M. Robinow, Yellow Springs, Ohio, for the results of the screening test.

In subsequent years, this patient developed the same deformities as his brother. He was admitted to the Hospital School for Handicapped Children at the age of 9 years (Fig. 1). At first, he was as apprehensive as his brother, but soon he demonstrated a good sense of humor and accepted his handicap. He was full of mischief and his reactions reminded the observer of the medieval court jester. His I.Q.'s after 2 examinations by the Wechsler Intelligence Test, were 79 and 81. He had considerable difficulty in walking and tired easily. The body weight was 16 kilograms, body length was 95 cm., sitting height 49 cm., armspan 91 cm., and the frontooccipital head circumference was 54 cm. He displayed the same type of disproportionate dwarfism as his brother. A detailed description can, therefore, be omitted. The slit lamp examination revealed corneal opacities of the entire corneal stroma. An audiometric examination revealed a hearing loss of 20 to 30 decibels in the range between 250 and 4,000 cycles per second. The tooth age was between 11 and 12 years as the second lower permanent molars were already erupted. There was generalized hypoplasia of the enamel. The cusps of the cuspids, bicuspids, and molars were unusually sharp and pointed. A systolic grade II murmur was heard in the third interspace on the left sternal border. The liver and spleen were not felt. The external genitals were normal. No abnormal neurological symptoms were present. Similar laboratory procedures were performed as in Case 1 and similar results were obtained. Reilly granulations were found in 60 per cent of the polymorphonuclear neutrophils of the blood and were present in the granulocytic row of the bone marrow. Serum calcium, inorganic phosphorus, alkaline phosphatase, cholesterol, and total lipids were normal. A 24-hour urine specimen contained 2.3 mg. 17-hydroxy steroids. A screening test for mucopolysaccharides in the urine was positive.* The electrocardiogram, electromyogram, and electroencephalogram were normal.

*Performed by Dr. Robinow.

The urine of Patient 1 was collected over a 20-day period and the mucopolysaccharide content was analyzed. The studies were done by Pedrini and Pedrini-Mille and will be the subject of a separate publication. The mucopolysaccharides were purified according to the method of Di Ferrante and Rich[41] and Meyer and associates.[42] A total daily excretion of 26 mg. of mucopolysaccharides was found in the urine of this patient as compared with 6 mg. in the urine of 2 normal children of the same age used as controls. A sample of the mucopolysaccharides was hydrolyzed with hydrochloric acid and the hydrolysate was run through a Dowex 50 × 8H+ to separate glucosamine from galactosamine according to Gardell.[43] The ratio of glucosamine to galactosamine in the mucopolysaccharides from the control urines was 1 to 10, whereas in our patient the ratio was 1 to 1. It appears evident from these data that our patient excreted very large amounts of mucopolysaccharides and the great proportion of glucosamine suggests the presence of some mucopolysaccharides other than chondroitin sulfate, possibly keratosulfate, although this compound has not yet been well identified. A small amount of chondroitin sulfate B was identified in one of the fractions of mucopolysaccharides in our patient, according to the method of Meyer and colleagues.[42, 44, 45]

The radiologic changes of the 2 cases were almost identical although the lesions were somewhat more severe in Case 1. The roentgenograms of the 2 cases will be described together.

The long bones of the upper extremities were more involved than those of the lower extremities. The humerus, ulna, and radius were short, coarse, curved, and irregularly tubulated. The epiphysial plates of these 3 bones were irregular. The olecranon and the coronoid process of the ulna were poorly developed and irregular. The distal epiphysial plate of the ulna was cupped. The distal epiphysial plate of the radius was at an oblique angle to the longitudinal axis of the radial diaphysis. The distal epiphysis of the ulna was not ossified. The remaining epiphysis of these 3 bones was very irregularly shaped and poorly ossified. Patient 1 had only 2, and patient 2 had 7 carpal ossification centers of

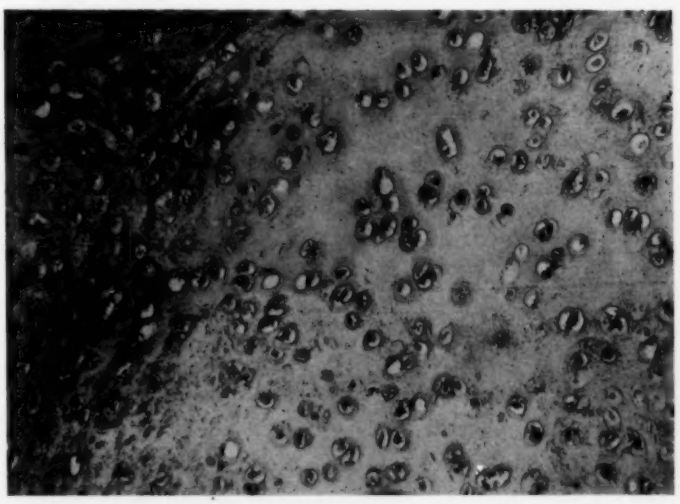

Fig. 7. Cartilage of iliac crest, showing large vacuolated chondrocytes distributed irregularly in unevenly strained matrix. The darkly stained area at left indicates a relatively high content of acid mucopolysaccharide. (Alcian blue. Original magnification ×250.)

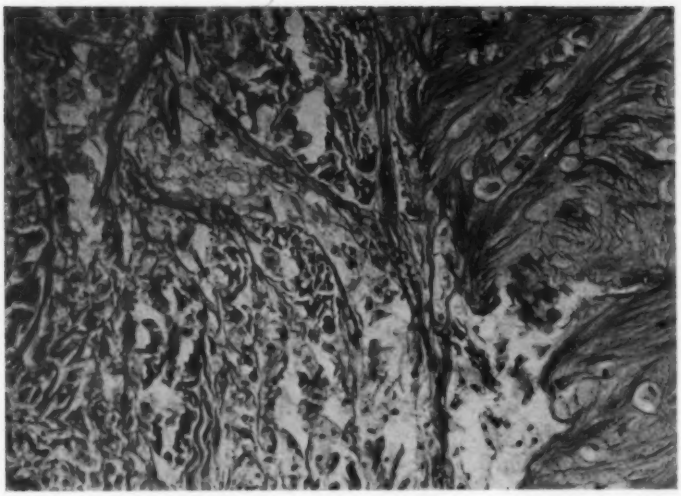

Fig. 8. Photomicrograph showing cartilage at right and perichondrium at center and left. Numerous large pale cells of histiocytic type are present in perichondrium. (Periodic acid-Schiff. Original magnification ×250.)

irregular shape (Fig. 2). The metacarpals and phalanges of the hand were short, coarse, and irregular. Some of these small bones had irregular epiphyseal plates and epiphyses (Fig. 3).

The diaphyses of the femur, tibia, and fibula were fairly straight and regularly outlined with almost normal tubulation. The metaphyses were considerably widened; the epiphyseal plates were irregular. The distal femoral epiphyses and the proximal tibial and fibular epiphyses were well developed. The proximal femoral epiphyses were small, flat, and irregularly ossified. The metatarsals and phalanges of the feet were coarse, short, and irregular.

The pelvic bones were coarse and somewhat irregular. The acetabular cavity was very wide and deep. A nonossified cap was present in the ischiopubic ramus (Fig. 4). A generalized platyspondyly was present in the spine (Figs. 5 and 6). The anteroposterior and transverse diameters were elongated. The intervertebral discs were relatively high.

Fig. 9. Cartilage defect near osteochondral junction. The large vacuolated cells filling the defect stain lightly with reagents designed to demonstrate mucopolysaccharides. (Periodic acid-Schiff. Original magnification ×125.)

Fig. 10. Higher magnificaton of cartilage defect showing fine strands apparently continuous with collagen of cartilage matrix at the periphery. Numerous thin-walled blood vessels are also shown. (Period acid-Schiff. Original magnificatior. ×250.)

The anterior part of the vertebral bodies was narrow and protruded forward like a tongue. However, the anterior part of the second and third lumbar vertebrae was hook shaped. The spinal transverse and articular processes were irregular. The head and neck of the ribs were also irregular. The ribs were wide and somewhat spatulous; their anterior endings were cupped. The clavicles were long and irregularly shaped. The scapulas were almost normal.

The skull revealed radiologically no major deformities. The sella was normal.

A biopsy, including skin, subcutaneous tissue, muscle, bone, and cartilage, was obtained from the iliac crest of patient 1 on April 1, 1960. The skin, subcutaneous tissue, and muscle were entirely normal to gross examination and presented no abnormal microscopic findings when stained with hematoxylin and eosin, periodic acid-Schiff, or Alcian blue after formalin fixation.

Fig. 11. Osteochondral junction at left, with defect extending into adjacent cartilage. The abundant vascular supply of the cartilage defect communicates with the marrow vessels through the defect in the osteochondral zone. (Period acid-Schiff. Original magnification ×125.)

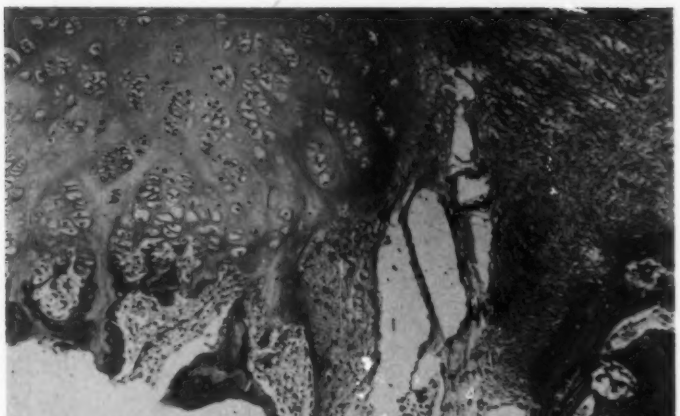

Fig. 12. Osteochondral junction, showing abnormal pattern of ossification. Irregular enchondral ossification is seen at the left. Intramembranous bone formation is seen at the right, adjacent to a large fibrovascular defect interrupting the osteochondral junction. (Hematoxylin and eosin. Original magnification ×150.)

The bone was invested with a cartilage cap up to 1 cm. in thickness. The subchondral plate and the underlying cancellous bone were moderately porotic and could be cut without difficulty with a razor blade. Sections cut from paraffin embedded tissue after formalin fixation and decalcification were stained with hematoxylin and eosin, periodic acid-Schiff reagent, and Alcian blue solution for microscopic examination. Extensive abnormalities were found in cartilage, perichondrium, and the zone of enchondral ossification. The cartilage was thickened and irregular, with large vacuolated chondrocytes throughout (Fig. 7). Many of these cells contained much Alcian blue staining material, comparable to the hypertrophied chondrocytes of the normal epiphysial plate. Cartilage matrix showed great irregularity of Alcian blue staining, with dense and pale staining areas intermingled in disorderly fashion. No conspicuous alteration of the periodic acid-Schiff staining pattern of cartilage was observed. Vacuolated cells, apparently of histiocytic type, were present in moderate numbers in the perichondrium (Fig. 8). The inner zone of the cartilage contained numerous well-defined, rounded, or ovoid areas 1 to 3 mm. in diameter in which closely packed cells with abundant pale-stained, finely vacuolated cytoplasm replaced cartilage cells and matrix (Fig. 9). Numerous fine strands with the appearance of collagen traversed these areas and extended into intact cartilage at the borders of the defects (Fig. 10). Several of the cartilage defects extended through the zone of enchondral ossification and contained blood vessels continuous with those of the marrow (Fig. 11).

The osteochondral junction was quite irregular and showed a very disorderly and abnormal pattern of enchondral ossification (Fig. 12). In some areas abortive columns of hypertrophied chondrocytes were seen, with a semblance of the normal sequence of calcification and ossification. These areas were interspersed with others in which fibrous or fibrovascular tissue was interposed between cartilage and bone, with foci of intramembraneous bone formation. The bony trabeculae were sparse and disorderly in

patterns, with many oriented obliquely or horizontally with regard to the plane of the zone of ossification.

The general characteristics of the histology of the skeletal lesions in this case were thus shown to be in part those described previously in Morquio's disease and in other respects were found to be similar to the lesions of Hurler's disease. The disorderly and abortive pattern of ossification is reminiscent of that described by Shelling,[34] and Einhorn and Rowntree[33] in Morquio's disease. The remainder of the findings, particularly the vacuolated cells infiltrating perichondrium and filling the defects in the inner zone of cartilage are in accord with a diagnosis of mucopolysaccharidosis such as Hurler's disease.

DISCUSSION

There is little doubt that the condition these 2 boys present is Morquio-Ullrich's disease. Its differentiation from Morquio's disease and Hurler's disease is based on the clinical and hematologic findings and the results of the histologic and the biochemical examinations.

Corneal opacities, hearing defects, and Reilly's granulations are not found in Morquio's disease, yet are typical findings in Hurler's disease. The presence of a heart murmur does not lend itself easily to differential diagnostic considerations. It may occur in Morquio's disease and Hurler's disease. In Morquio's disease it may be due to the thorax deformity altering the position of the heart; in Hurler's disease it can be due, in addition, to the involvement of the valvular apparatus. The external appearance, the physiognomy, and the roentgenologic features of the bones are Morquio's disease–like and allow us to rule out Hurler's disease, though according to Lamy[5] platyspondyly has been found in Hurler's disease. In a series of about 30 cases of Hurler's disease observed by one of the authors (unpublished cases[4, 46-48]) we never found platyspondyly. The combination of the clinical and hematologic data allows, therefore, Hurler's disease and Morquiro's disease to be ruled out and suggests the diagnosis of Morquio-Ullrich's disease.

The histologic findings obtained by the biopsy examinations of the iliac crest of Patient 1 reveal lesions which have been found in Morquio's disease. However, hypertrophy and vacuolization of the chondrocytes and other cells found in the defects in the inner zone of the cartilage are comparable with the alterations found in Hurler's disease. The combination of these histologic findings is suggestive of an entity separate from Hurler's disease and Morquio's disease.

Increased amounts of mucopolysaccharides were found in the urine of one of the 2 patients indicating the presence of a mucopolysaccharidosis. Besides chondroitin sulfate B which is usually found in Hurler's Disease another mucopolysaccharide, containing large amounts of glucosamine, was found in the urine of our patient. A glucosamine containing mucopolysaccharides (heparitine sulfate) is found in Hurler's disease as well, though it represents only a minor component of the total urinary mucopolysaccharides in Hurler's disease.[29] The glucosamine-containing fraction in the urine of our patient represents the major component of the urinary mucopolysaccharides. Its nature has not been fully identified. There is some evidence indicating that it is a keratosulfate. The urinary mucopolysaccharide of our case differs, therefore, from those found in Hurler's disease. No increase of urinary mucopolysaccharide has so far been reported in Morquio's disease.* Thus, the biochemical studies of the urine of one of our patients also indicate the presence of a pathologic condition other than Hurler's disease or Morquio's disease.

In conclusion, it can be stated that the children reported upon in this publication present a condition which, on the basis of the clinical and hematologic findings, can be diagnosed as Morquio-Ullrich's disease.

*Pedrini (personal communication) found in one case of Morquio's disease in a 2-year-old girl increased amounts of a mucopolysaccharide which is probably not chondroitin sulfate B. The substance is still under analysis. Dorfman (personal communication) examined the urine of one child with Morquio's disease and did not find increased amounts of mucopolysaccharides.

For the first time, histologic and biochemical data are presented which support the assumption that Morquio-Ullrich's disease is a mucopolysaccharidosis different from Hurler's disease.

SUMMARY

Two brothers with Morquio-Ullrich's disease are presented. The 2 boys were disproportionate dwarfs with an I.Q. in the low normal range and Morquio's disease–like skeletal changes and external appearances. In contrast to the usual findings in Morquio's disease, each boy had corneal opacities, a mild hearing defect, and a cardiac murmur. Reilly granulations were found in the granulocytes of the peripheral blood and in the granulocytic row of the bone marrow. A bone biopsy of one of them revealed evidence of mucopolysaccharidosis. In the urine of one of the boys, increased amounts of mucopolysaccharides were found which differ in their qualitative composition from the mucopolysaccharides found in the urine of patients with Hurler's disease. Morquio-Ullrich's disease can, therefore, be considered as a mucopolysaccharidosis. Its differential diagnosis from Hurler's and Morquio's disease is discussed.

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Subepicranial hydroma

A complication of head injuries in infants and children

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A BENIGN complication of head trauma in children may cause concern is the appearance of a soft fluctuant swelling beneath the scalp within hours or days after the injury, often first recognized by the mother when she combs or washes the child's hair. A fall may have occurred from a crib, chair, or bassinette without apparent sequelae until the cystic accumulation appears. Early in its course, the lesion may be regarded as a simple hematoma and dismissed. However, it may increase remarkably in size within a brief interval of time, and because of this roentgenograms of the skull are taken. Most frequently, a linear fracture is found in the parietal area traversing a suture line (Fig. 1). After 1 or 2 weeks, the swelling disappears spontaneously, leaving no residual signs of injury.

The swelling of the scalp discussed here is essentially a collection of spinal fluid with or without an admixture of blood beneath the epicranial aponeurosis (galea). It has been improperly referred to as a transient false meningocele[1] and spurious meningocele[2] because of the nature of the contained fluid and its connections with the subarachnoid space. Confusion has occurred in relation to cephalhematomas and depressed fracture. The proper designation is that suggested by Solomon,[3] namely, subepicranial hydroma.

The dura is maximally adherent at the various sutures, and consequently is torn as the fracture occurs. The laceration extends into the subarachnoid space. Spinal fluid escapes through the dural defect, traverses the fracture line and the tear in the pericranius (periosteum), and accumulates in the subepicranial space (Fig. 2).

The fluid may not appear beneath the scalp until after an interval of hours to days. A factor delaying the leakage might be the rate of absorption of blood clot in the fracture line. The linear defect subsequently may widen as a result of a transient increase in intracranial tension facilitating the transfer of cerebrospinal fluid into the subepicranial space. The fluid does not gather beneath the pericranium. Hence it is not, as in a cephalhematoma, confined to the boundaries of a single bone where the pericranium and a suture line fuse (Figs. 2 to 5). Hydromas have not been found

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in the perinatal period and, therefore, cannot be confused with cephalhematomas. By dissecting through the subepicranial areolar tissue, fluid may accumulate over a wide area covering the entire vault (Fig. 3). The only restrictions are the attachments of the epicranius to the external occipital protuberance and superior nuchal line posteriorly and to the superciliary ridges and subcutaneous tissues over the eyebrows and nose anteriorly.

The injury is most often sustained in the parieto-occipital region. Usually the fracture appears in an area of transmitted stress in the parietal bone. Fortunately, the absorptive capacity of the resilient calvarium as it fractures reduces the impact energy at the surface of the brain. Hence, signs of serious cerebral dysfunction such as unconsciousness and focal abnormalities are infrequent. Transient drowsiness and vomiting may occur. These symptoms usually subside before the fluid escapes.

The scalp overlying the cystic collection is nontender, intact, and may be transilluminated. A doughy rim sometimes circumscribes the area of swelling especially in the earlier, more confined lesions and may suggest a depressed fracture much as that in hematomas of the scalp with central softening. Hydromas can be differentiated from hematomas by the absence of local tenderness, contusion, and discoloration of the skin. A hematoma is firm and has a characteristic "snowball crepitus." As it subsides, it leaves the scalp indurated and often attached to the underlying structures, since it may involve all of the many layers of the scalp.

Roentgenograms of the soft tissue of the scalp tangential to the area of swelling may reveal irregular densities in the presence of a hematoma. Only a uniform, ground-glass clouding is seen in the hydroma (Figs. 3 and 4).

We have had the opportunity to observe 13 children with subepicranial hydroma associated with cranial injuries. There were 6 boys and 7 girls whose ages ranged from 5 months to 13 years. Five patients were under 1 year of age. The children had all sustained falls except one, who had been struck by a baseball bat. The only severe injury occurred in a rapid deceleration injury when the infant fell from a moving bicycle and was unconscious for almost 3 days. The other patients were never unconscious and suffered no more immediate reaction than transient drowsiness and occasional vomiting. Swelling of the scalp was observed as early as 3 hours after injury. In 6 patients it was recognized during the first day, in 4 on the third day, in 2 on the fourth day, and in 1 on the sixth day after the traumatic event. The fracture line was evident in roentgenograms in 10 patients,

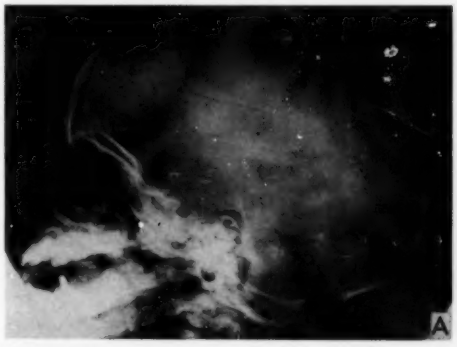

Fig. 1*A*. Linear fracture in the parietal bone extending into the coronal suture in a 3-year-old boy with a subepicranial hydroma.

Fig. 1*B*. Linear fracture in the frontal bone crossing the coronal suture and extending into the parietal bone in a 10-year-old child with a frontoparietal hydroma.

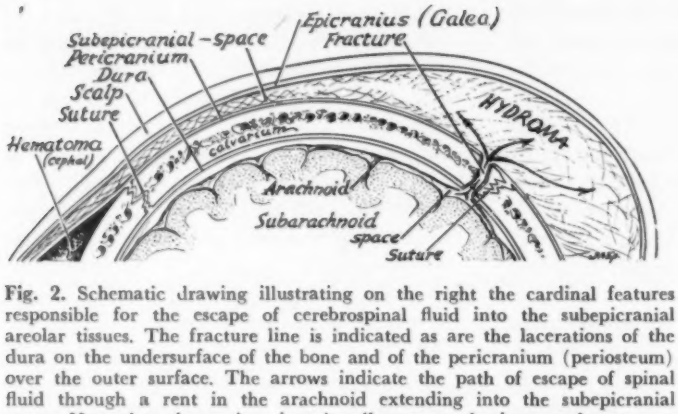

Fig. 2. Schematic drawing illustrating on the right the cardinal features responsible for the escape of cerebrospinal fluid into the subepicranial areolar tissues. The fracture line is indicated as are the lacerations of the dura on the undersurface of the bone and of the pericranium (periosteum) over the outer surface. The arrows indicate the path of escape of spinal fluid through a rent in the arachnoid extending into the subepicranial space. Note that the pericranium is adherent to the bone and suture at the site of fracture. The fluid accumulates between the pericranium and the overlying epicranius. On the left, a cephalhematoma is illustrated. The collection of blood lies beneath the pericranium and is confined by the attachment of this layer to the suture line.

the parietal bone being involved in each case; in 3 of them the frontal bone was also involved (Fig. 1). The fracture extended into either the coronal or sagittal sutures. In only the severely injured patient did it extend into the base. The swelling was unilateral in all except one patient in whom the swelling encircled the head (Figs. 3 and 4). Transillumination was positive in one child. In 2 infants straining caused the soft mass to bulge. No demarcating borders or margins, other than the natural supraorbital and occipital boundaries, were noted in 2 of the most extensive hemicranial accumulations. The parietal area was the initial site of swelling even though the occiput was most often the area of impact. There were no focal neurological changes except for a mild, evanescent facial weakness with hyperreflexia in one child. Angiography performed in the seriously concussed patient was negative. The electroencephalogram showed transient slowing of a nonfocal character in this case. The hydroma disappeared spontaneously in all by the sixteenth day, signs of resolution being observed after 7 to 9 days. The firm marginal elevation when pres-

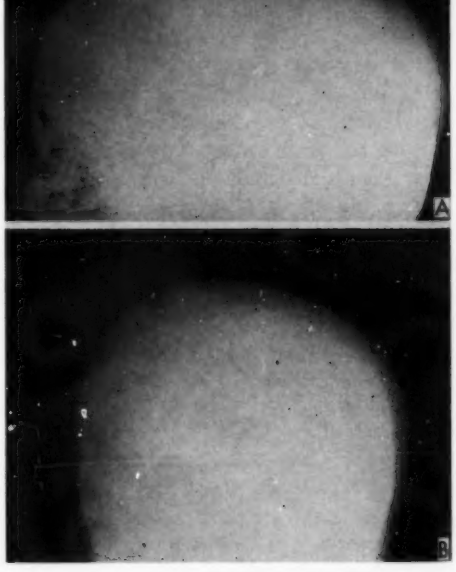

Fig. 3. Soft tissue roentgenogram showing the uniform, soft, ground-glass clouding typical of the hydroma encircling the entire calvarium in this child.

ent disappeared quickly once the fluid absorbed, the scalp remaining freely movable and normal in all respects. Lumbar puncture was not performed, and the danger of introducing infection contraindicated aspiration of the fluid in any patient. No therapy was needed except to provide reassurance for the parents. There were no sequelae and no patient developed symptoms or signs of leptomeningeal cyst formation. Although there was apparently no instance in which a hematoma of the scalp was combined with the subepicranial hydroma, such lesions may coexist. An appreciation of the characteristics of each lesion as described above will aid in the management of such patients.

DISCUSSION

Brozsan and Brennan[1] in a comprehensive review of "transient false meningoceles" in 1940 presented 29 patients seen over a 10-year period. In 4 of them aspiration of the cystic mass had been performed; clear cerebrospinal fluid was removed in 2, and spinal fluid mixed with blood was obtained in the others. The fluid reaccumulated after tapping, and the procedure was not recommended. Fractures were of the indirect type and seldom involved the base. They observed temporary changes such as ecchymosis of the eyelids and transient palsies of eye muscles with diplopia. The ages of their patients ranged from 1 to 13 years with the exception of 2 who were 17 and 18 years, respectively. The swelling was noted soon after the accident in 17 cases and was delayed in the remaining 12, one being discovered as late as 14 days after the injury. The mass was bilateral in 2 patients. The fluid collection lasted from 2 to 28 days. Roentgenologic evidence of fracture was encountered in 27 of the children. One must be aware that a fracture may exist without roentgenographic verification. In 14 patients, only mild clinical manifestations were observed, moderate in 10 and severe in 5. The only death occurred in a child with a purulent meningitis in whom a direct communication was observed at autopsy between the intra- and extracranial compartments.

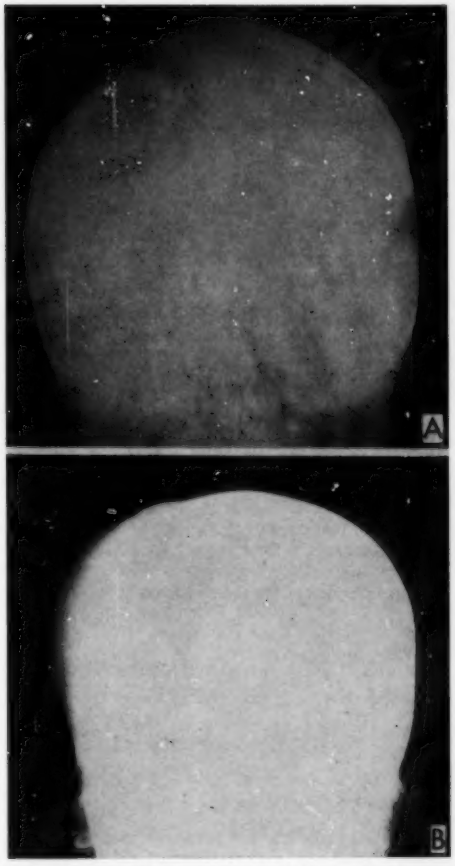

Fig. 4. *A,* Hydroma in the right parietal area. Note the soft outer margins and the clearly defined outer table of the skull. *B,* Calcified cephalhematoma in the left parietal region. The shadow has greater density, the distinct outer layer of the scalp is sharply defined, and the underlying calcified-ossified mass has an irregular outer margin near the vertex.

Solomon[3] described an injured child who developed a soft swelling in the left parietal area that subsequently covered the entire calvarium, including the eyelids. The swelling disappeared spontaneously after 16 days without further difficulty.

Voris[2] decided that aspiration is unnecessary, since spontaneous closure of the dural defect always occurred eventually. As long as the tear in the dura remains open, it is

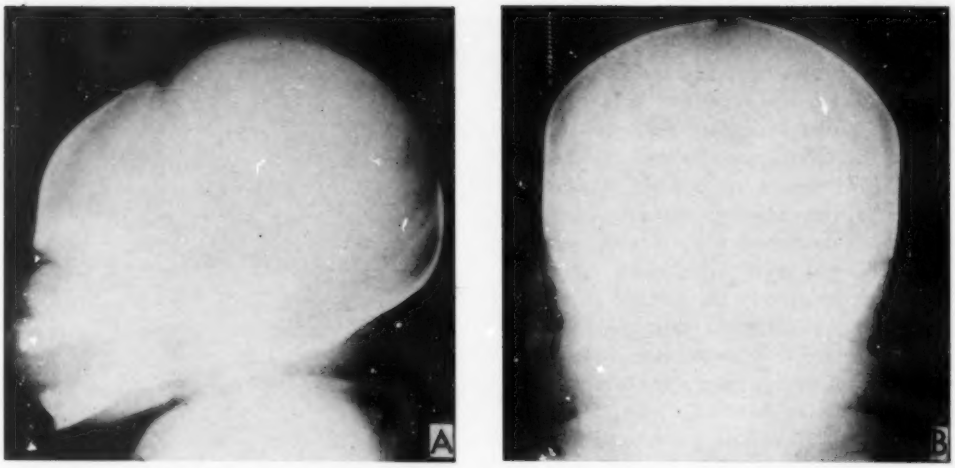

Fig. 5. *A*, Newborn infant with a cephalhematoma. The clearly defined double density overlying the skull dips in the mid-vertex area and (*B*) conforms to the attachment of the periosteum to the suture lines. This roentgenogram should be compared with those of the subepicranial hydromas in Figs. 3 and 4, *A* where the shadow of the fluid layer blends into that of the underlying scalp with a soft, blurred margin, possibly caused by the lesser density of spinal fluid than that of blood.

inevitable that fluid will traverse it and accumulate beneath the epicranium thus making the procedure useless as well as hazardous.

SUMMARY

The accumulation of spinal fluid beneath the epicranius as a soft, fluctuant mass is a complication of head injury with fracture in children. The swelling is nontender and is usually not associated with any significant evidence of brain injury. It appears within hours or days after the trauma, which usually is of the blunt type. Once apparent, the swelling may increase dramatically in size and, at times, envelop the entire hemicranium. A marginal rim is present in some of the more restricted collections, usually in the parietal region. Roentgenograms disclosed linear fractures in 80 per cent of the cases, which are most often in the parietal area and often cross a suture line. The dura-arachnoidal tear occurs at the fracture line and permits the escape of cerebrospinal fluid into the subepicranial areolar tissue. The fluid is absorbed spontaneously; aspiration is contraindicated because of the possibility of introducing infection. No treatment is required.

We would like to thank Albert Florman, M.D., for his permission to use 2 cases in this report from the Pediatric Service of the North Shore Hospital, Manhasset, N. Y.

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Mesenteric vascular occlusion in infancy and childhood

Review of the literature and report of an additional case

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A CAREFUL search of the literature concerned with mesenteric vascular occlusion discloses only a few cases occurring in infancy and childhood. Table I lists these cases and the data, where given, pertinent to them.

Because the lesion is so rare in the younger age group, the following case is recorded.

A white female infant was born 6 weeks prematurely at the Thomas M. Fitzgerald Mercy Hospital at 2:44 A.M. on May 18, 1960. The infant was delivered after an 8-hour labor from the left occipitoanterior position. At the time of delivery the amniotic fluid was described as deep green. Both parents were Rh positive and the pregnancy was uncomplicated except for a minor infection of the urinary bladder which was treated with sulfisoxazole. The mother is 30 years old, has 4 living children, and a history of one abortion at 6 weeks' gestation.

The initial physical examination revealed a white female infant weighing 6 pounds, 8 ounces, and having "a lusty cry, good color, fair sucking reflex, Moro reflex, a heart rate of 132 per minute, and a respiratory rate of 20 per minute." When she was 4 hours old, grunting respirations were noted, and at 8 hours cyanosis and apnea developed and the abdomen was noted to be distended and tight. There were no signs of convulsion. The child was in an Isolette and oxygen was administered at a rate estimated to provide 3 L. per minute. A roentgenogram of the abdomen was reported as showing "no significant intestinal gas pattern." A hemogram disclosed 16.5 Gm. of hemoglobin with 5.6 million red blood cells and 22,750 white blood cells per cubic millimeter, 51 neutrophils, and 49 lymphocytes. Penicillin and streptomycin therapy was started, and gastric aspiration was begun. The condition remained about the same until approximately

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20 hours of age at which time cyanosis returned and the infant became listless. At this time vomiting of brown-flecked material began. She was given ACTH, epinephrine, a corticosteroid, and antibiotics in massive doses. She did not respond to the therapy, and died at 11:35 A.M. on May 19, 1960, after some 33 hours of life.

At autopsy the body weighed 2,800 grams and measured 32.5 cm. from crown to rump. There was marked livor mortis of the skin on the head and neck, but no other external changes were noted. No anomalies of the heart or great vessels were noted, and both the foramen ovale and ductus arteriosus were patent. There was partial atelectasis of both lungs with brown-stained fluid in the lower trachea and main-stem bronchi. The leaves of the diaphragm were elevated to about the fifth interspace bilaterally. When the peritoneal cavity was opened foul-smelling, bloody fluid was released and loops of the distended small bowel welled from the incision. The entire small bowel distal to the ligament of Treitz, the cecum, and the first portion of the ascending colon were hemorrhagic, thin walled, and friable. The involved bowel contained bloody mucoid material and clots of blood. When

Table I. Analysis of previously reported cases of mesenteric vascular occlusion in infancy and childhood

Author	*Year*	*No. of cases*	*Sex*	*Age*	*Vessel involved*	*Cause*	*Clinical diagnosis*	*Result*
Klob[1]	1859	1	F	8 days	Superior mesenteric artery	Embolus from ductus arteriosus	Not given	Death
Jackson, Porter, and Quinby[2]	1904	3	Not given	1 month 5 years 8 years	Not given	Not given	Not given	Not given
Trotter[3]	1913	4	Not given	5 years to not more than 10 years	1 arterial, 2 venous, 1 coexistent (venous and arterial)	Not given	Not given	Not given
Frank[4]	1923	3	1. M 2. M	8 years 10 years	"Mesenteric vessels"	1. Unknown 2. Endocarditis	1. Peritonitis 2. Acute appendicitis	1. Death 2. Living 3 years after operation
			3. M	12 years		3. Obstructing band	3. Peritonitis	3. Death
McClanahan[5]	1926	1	M	4 months	"Mesenteric vessels"	Unknown	Intestinal obstruction	Death
Feldstein and Goldstein[6]	1936	1	F	6 years	Superior mesenteric artery	? Chronic mastoiditis with sepsis	Ulcerative process in colon	Death
Johnson[7]	1940	1	M	2 months	"Mesenteric vein"	Unknown	Intussusception	Death
Morison[8]	1945	1	M	18 days	Inferior mesenteric artery	Thrombosis of aorta	Not given	Death
Rothschild, Storck, and Myers[9]	1953	1	M	Newborn	Superior mesenteric artery	Unknown	Partial obstruction of small bowel	Death
Schaffer[10]	1960	1	F	5 weeks	"Mesenteric vessels"	Mesenteric cyst	Not given	Death

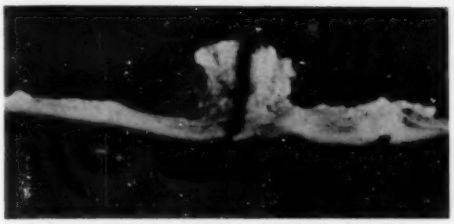

Fig. 1. Longitudinal section of the abdominal aorta through the stump of the superior mesenteric artery showing occlusion of that artery by an antemortem blood clot.

the cecum was freed there was a small collection of retrocecal, purulent material that extended upward to discolor the capsule of the lower pole of the right kidney. There was no evidence of a cecal perforation and no evidence of appendiceal inflammation. The remainder of the ascending, transverse, and descending colon showed no changes and contained sticky, dark green meconium. The anus was patent. The abdominal aorta was removed and opened along the posterior wall. This revealed a "T" shaped, light red-brown, friable blood clot in the lumen of the aorta. The clot measured 1.1 cm. long by 0.5 cm. across the middle. The leg of the "T" extended into the superior mesenteric artery and occluded it completely (Fig. 1). No other gross changes were noted in the body; the brain was not examined.

Microscopically, an organized blood clot was seen in the aorta; it extended into and occluded the superior mesenteric artery. Partial atelectasis of both lungs was noted with the presence of foreign material in a number of the alveoli. The small bowel and cecum were gangrenous. Extramedullary hematopoiesis occurred in the liver. Both of the kidneys were congested, and there was acute inflammation limited to the capsule of the right kidney.

DISCUSSION

A review of the literature indicates that mesenteric vascular occlusion occurs slightly more often in men than in women, a 3 to 2 ratio of incidence. In the few pediatric cases reported a similar sex differential is noted.

A similar ratio is observed in the incidence of mesenteric arterial occlusion when compared with mesenteric venous occlusion. The former are limited almost entirely to the superior mesenteric artery with only a few reports of inferior mesenteric artery occlusion. Gallavardin[11] explains this by pointing out 3 anatomic differences between these 2 arteries: (1) the higher position of the superior mesenteric artery makes earlier interception of emboli more likely; (2) the caliber of the superior mesenteric artery is more than twice that of the inferior mesenteric artery; and (3) the superior mesenteric artery runs almost parallel to the aorta, whereas the inferior mesenteric artery leaves it at an abrupt 45 degree angle.

Thus, it is easy to understand why emboli are so frequently the cause of arterial occlusions. The more common sources of such emboli are the pulmonary veins, the left auricular appendage, the mitral and aortic valves, and the left ventricular endocardium. In infants the presence of a patent foramen ovale or a patent ductus arteriosus can explain an embolus from the circulation on the right side. In contrast, venous occlusion is caused exclusively by a thrombosis which most frequently results from an abdominal inflammatory disease, such as appendicitis, strangulated hernia, malignancy, pelvic disease, or pregnancy.

The classical symptoms of lassitude, mental depression, anorexia, vomiting, bloody diarrhea, abdominal pain, subnormal temperature, and abdominal distention followed by death in 12 to 48 hours was duplicated by Litten[12] in animals in which he occluded the superior mesenteric artery with ligatures.

Although radiographic studies may show dilatation of the bowel,[13-15] Buckstein[14] points out that if the gangrenous bowel is filled with bloody exudate it may eliminate this finding. Laboratory studies are usually noncontributory. Therefore, the clinician must base his diagnosis almost exclusively on the clinical picture, and, if the patient is to be

saved, procrastination by the surgeon must be avoided. Frank[4] points out the urgency of surgical intervention, and states that "wasting valuable time and probably sacrificing life by laboratory and other useless diagnostic refinements," partially explains the high death rate from this disease. Modern surgery and anesthesiology, with the aid of anticoagulant therapy, have considerably reduced the number of deaths.

The etiology of the vascular occlusion in our case is entirely unknown. The sudden onset of symptoms suggests that it was an embolic occlusion, but our studies failed to demonstrate the source of such an embolus.

SUMMARY

The literature concerning mesenteric vascular occlusion in infancy and childhood has been reviewed and an additional fatal case reported.

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Meprobamate in the management of enuresis

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IN SPITE of continuous medical interest, enuresis still represents one of the most commonly encountered, yet least understood, problems of childhood psychopathology. It ranks high among these conditions for which the widest variety of methods for treatment are employed. There have been few published reports of the application of the newer psychopharmacologic agents to the management of enuresis, and those reported[1-6] have failed to meet criteria for a well designed, controlled investigation.

A clinical study of the use of meprobamate in the treatment of enuresis was undertaken in the pediatric outpatient department of The Johns Hopkins Hospital. The design of the investigation was based on the following considerations. Enuresis is an exceedingly common disturbance. Bloomfield and Douglas[7] observed it in 12.4 per cent of 4,294 children at 4 years of age and in 7.3 per cent of them at 8 years of age; others[8-10] report incidences varying from 20 to 26 per cent at 4 years of age. Considerable attention has been focused on the role of organic factors in enuresis. Cohen[11] stressed the presence of urologic lesions in adult enuretic males. Stockwell and Smith[12] and Brodney and Robins[13] described urologic abnormalities in about 15 per cent of enuretic children, and Fisher and Forsythe[14] in 50 per cent of enuretic children resistant to medical treatment. Campbell[8] demonstrated an organic basis for enuresis in one half to two thirds of children not responding to conservative medical management but added that this number would account for not more than 5 per cent of all cases and that many children apparently stop wetting the bed during urologic treatment in order to avoid further instrumentation. Bakwin[9] believes that only a small percentage of cases are organic in origin and that treatment for the oft-coexisting balanitis and phimosis may result in a "psychic cure." Halgren[15] in a genetic study emphasized the minimal role of physical causes, and Little[16] concurs that uropathies are relatively infrequently associated except perhaps in cases of severe, diurnal enuresis, adding that cessation of wetting with correction of concomitant urologic abnormalities does not establish a causal relationship between lesion and symptom.

The above opinions are based mainly on cystoscopic and radiologic investigations, although electroencephalographic and cysto-

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Wyeth Laboratories generously supplied the meprobamate and financial support to make this study possible. Research project No. M.32.185, Johns Hopkins University.

metric studies have also been utilized. Michaels and Secunda[17] found that among children with a variety of neurotic traits the highest incidence of abnormal electroencephalographic tracings (consisting primarily of "waves of slow rate") was in those who were enuretic. Turton and Spear[18] obtained 51 abnormal tracings among 100 enuretic children; in 23 the tracing was suggestive or pathognomonic of epilepsy, and in 28 there was an "immature pattern." Cystometric studies by Campbell[8] revealed no difference in pressure readings between enuretic and normal subjects, but Cohen's[11] study indicates that discomfort and desire to urinate arises at an average pressure of 45 cm. of water as compared to an average of 70 to 80 cm. in normal children. It would appear from these considerations that in the vast majority of instances organic factors cannot be clearly demonstrated, and in the exceptional cases with an organic basis, the diagnosis can be elicited by careful history, physical examination, urinalysis, and such specific studies as indicated. To other authors enuresis appears to be an emotional symptom of varying severity[19, 20] but it is rarely the only indication of such a disturbance.[16, 21-23] Little[16] believes enuresis is related to a disequilibrium in the parent-child relationship, most often stemming from premature toilet training with which frustration and anger are associated. Campbell[8] was impressed with the role of parental discord in which anxiety was transmitted to the child, whereas Kanner[10] stressed the importance of maternal oversolicitude which results in infantilization. He also emphasized that once enuresis was established, other emotional manifestations and personality changes were bound to follow. The role of inadequate basic toilet training in enuresis is mentioned prominently by many authors.[8-10] Many psychoanalytic formulations have been proposed. Despert[24] and Menninger[25] have both stressed the aggressive, hostile meaning of enuresis as a symptom, whereas others have viewed it as a monosymptomatic manifestation of hysteria. Freud[26] in early writings viewed enuresis as urethral eroticism psychologically equivalent to nocturnal emissions. Gerard[19] in a psychoanalytic study of 65 enuretic children viewed the symptom as resulting from castration anxiety. The males were passive, retiring, and self-depreciatory, resulting from an identification with the mother, while enuretic females identified with the father were active, proficient, and independent personalities. Kanner[10] in reviewing studies of personality patterns of enuretic children found them to be so diverse as to defy usefulness but was impressed by their general immaturity. It is of interest that Schachter and Cotte[27] found no particular Rorschach patterns in children with enuresis. Whereas the above refers essentially to persistent enuresis, Campbell,[8] Kanner,[10] and others described the commonly seen appearance of enuresis as a regressive symptom in previously trained children suddenly placed in an acute stressful situation such as dethronement by a newborn sibling. Even those who would view enuresis as an inherited condition, in which difficulty exists inhibiting detrusor contraction after a certain volume of distention, have all stated that only a potentiality is genetically transmitted and that this becomes symptomatic only under certain environmental conditions.[8, 15, 28] Kanner[10] feels no adequate proof exists to substantiate that it is not tradition which is transmitted rather than genes.

By means of the enuretic symptom the child attempts to resolve, albeit ineffectively, some anxiety provoking problem and will, as a result, manifest less overt tension. Schwartz[29] views enuresis as a safety valve for the escape from many intolerable situations. It would seem that any therapeutic undertaking might revive some of the basic problems and associated anxiety. To quote Kanner,[10] "Such is the integration of motives and effects in the personality of enuretic children that the therapeutic improvement of the effects touches directly upon some of the basic conflicts which have given rise to the enuresis." Therefore, a tranquilizer might play a significant role in treatment. In view of the marked frequency of this symp-

tom, management falls upon the shoulders of the pediatrician and family physician. Intensive psychotherapy is rarely available, which further enhances the important contribution which an effective drug might make.

METHOD OF STUDY

One hundred children between the ages of 5 and 14 years with enuresis sufficiently severe to have the complaint mentioned to a physician were studied. Referrals were made through the outpatient department of this hospital, the Baltimore City Grade School Health Service, and practicing physicians. Children with known or diagnosed organic uropathies and/or severe mental retardation were excluded.

A history, physical examination, and urinalysis were done in each case. The history included *(A)* a study of the presenting symptom (duration, onset, association of traumatic events, diurnal or nocturnal, frequency, familial history of enuresis, parental attitudes and actions), *(B)* patterns of growth and development, *(C)* family social patterns, and *(D)* manifestations of anxiety and maladjustment. Each evaluation lasted 45 to 60 minutes and it was performed by the same physician with both parents and child in the room.

The 100 cases were divided by random selection into two groups of 50 children each, the one receiving meprobamate, the other a placebo. The capsules were identical in appearance and were coded by another member of the department. The code was unavailable to the experimenter until after the completion of the study. Meprobamate was dispensed in 200 mg. tablets with a dosage schedule of 200 mg. 3 times a day for patients aged 5 to 9 years, and 3 doses per day consisting of 400 mg., 200 mg., and 400 mg. respectively for children 10 to 14 years of age. The doses were constant and were not increased in children in whom no improvement was evident.

After evaluation of the total situation, an interpretation was given to the family in the child's presence. Although varied to meet individual needs, the usual approach was somewhat as follows: "Mrs. Jones, the physical examination and urinalysis show your child to be physically healthy. We find that, since we have eliminated physical disease, this is an emotional symptom. For various reasons these children tend to be tense and anxious. This tension can show itself outwardly (mention any pertinent examples in the patient) or inwardly in the workings of the organs. You know from your own experience that when you are upset your stomach tightens or your bowels grumble. So it is for your child, except that for him it may be the bladder which tightens. The bladder is a thick muscular bag which can enlarge to hold great quantities of urine. However, when tense, it fills incompletely and has to empty suddenly, resulting in frequent and urgent voidings. At night conscious controls are lessened and, with the child's normal desire to sleep, this bladder irritability is quite disturbing. With time the child breaks off contact with the bladder and signals are no longer received by the mind."

At this point some idea is presented of why the anxiety is present if it is apparent from the evaluation or, if not, some vicissitudes of a child's growing up are discussed. Medication is then proposed as a way of relaxing the bladder, and it is added that because at present no functioning connection exists between bladder and mind at night, even should the drug be successful, time will be required for the child to reestablish functional control. The medication is then offered the child with some supportive statement indicating expectation that it will be effective and with confidence in his ability to control the wetting with time. A request

Table I. Number of nights bedwetting occurred per week

Initial	*After treatment*	*Designated level of improvement*
7	0	Cured
7	1 or 2	Greatly improved
7	3, 4, or 5	Improved
7	6 or 7	Unchanged

Table II

Drug				*Placebo*			
Case No.	*Initial frequency*	*Final frequency*	*Change in frequency*	*Case No.*	*Initial frequency*	*Final frequency*	*Change in frequency*
1	4	4	0	4	7	2	5
5	5	2	3	8	7	2	5
6	7	0	7	9	7	0	7
11	7	1	6	12	7	0	7
16	7	7	0	13	7	4	3
20	7	2	5	17	7	7	0
22	7	2	5	18	7	4	3
24	7	7	0	19	7	5	2
28	7	2	5	21	7	0	7
30	7	2	5	25	7	4	3
31	7	7	0	26	7	1	6
33	7	3	4	27	7	1	6
35	7	3	4	32	7	7	0
42	7	5	2	37	7	5	2
53	5	2	3	43	7	1	6
55	7	7	0	45	7	6	1
57	7	2	5	48	7	6	1
59	7	1	6	56	7	0	7
61	7	3	4	62	7	1	6
64	7	7	0	63	7	7	0
69	7	7	0	65	4	2	2
73	7	2	5	67	7	2	5
75	7	2	5	72	7	2	5
76	7	1	6	74	4	2	2
79	7	5	2	77	7	2	5
80	7	3	4	78	7	4	3
81	7	0	7	82	7	7	0
83	7	4	3	85	4	0	4
86	7	1	6	87	7	4	3
88	7	7	0	89	7	7	0
91	7	1	6	92	4	0	4
93	7	7	0	94	7	7	0
95	4	2	2	96	4	4	0
97	7	0	7				
No. of cases 34 Total change = 117 M = 117/34 = 3.44				No. of cases 33 Total change = 110 M = 110/33 = 3.33			

is made for the cessation of recrimination and bribing ("as these haven't worked anyway") and to replace them with praise and encouragement. It is also requested that there be no restriction of fluids and no awakening at night ("let's keep as many things as normal as possible").

The patients were seen at intervals of 2 weeks, 1 month, 2 months; and 3 months (final evaluation). At each succeeding visit the examiner briefly discusses the progress made. Regardless of what is reported, support and some area of encouragement is found and a new supply of medication is given.

RESULTS

Only those 67 patients who completed the treatment were included in the final analysis. A study of the 33 not completing the treatment revealed as many to be responding well to treatment as poorly, and as many on the drug as on placebo. There appeared to be no other significant trends which would make them an important group.

Improvement rating utilized the following scheme. Initial enuresis was stated as the number of nights bedwetting occurred in a week which is, of course, an average expressed by parents. At each revisit a similar frequency was obtained and the change in fre-

quency of enuresis was derived, which represented the progress or worsening between the visit and initial severity. A final frequency was then fitted into the following scheme.

With the assumption that the child originally wet 7 nights, as was the case in the vast majority of patients (58 of 67), and knowledge of the final frequency, Table I was devised.

With the above as the general design for evaluating progress, we may now turn to the results and conclusions. There was no statistically significant difference in improvement between the group that received the drug and the group that received the placebo (Table II).

In spite of a lack of specific pharmacologic effect of meprobamate on enuresis, the total treatment plan was therapeutic in an appreciable number of cases as shown by Table III. Thus, in 45 per cent (cured and greatly improved) a result was obtained which would generally be considered quite satisfactory by most clinicians, and in approximately 75 per cent (cured, greatly improved, and improved) some degree of breakup of the chronic pattern was accomplished.

There was no statistically significant difference in improvement between males and females taken as a group or between the sexes in either therapy group.

When all degrees of improvement are considered (including "0 improvement"), improvement is not correlated with age. However, when the 16 children who did not have any improvement are removed from the analysis, the correlation between age and improvement is –0.25 ($p < .10 > .05$). This means that the younger the child the more apt he is to improve greatly. This observation poses the question of what is unique about the 16 children who showed no improvement; the data indicate nothing special about these.

Initial severity of bedwetting is neither correlated with the child's age nor related to degree of improvement.

All subjects were sorted into the following categories based on historical data elicited during the diagnostic interview: *(A)* those in whose family there is a history of enuresis; *(B)* those whose history revealed a serious perinatal, developmental physical illness or handicap; *(C)* those who present *both (A)* and *(B)*, and *(D)* those who present *neither (A)* nor *(B)*. If the question is asked, "Do these four groups differ in terms of response to treatment?" the answer is, "No." But if we start out with the a priori hypothesis that *(D)* will improve most, *(A)* next, then *(B)*, and then *(C)*, the data do support this hypothesis. (This utilizes A. R. Jonckheere's principle of "A Distribution Free K-Sample Test against ordered alternatives.")

Degree of anxiety and maladjustment is not related to improvement in the drug group, placebo group, or in the total study population.

ADDITIONAL OBSERVATIONS NOT DIRECTLY RELATED TO THE STUDY

In 47 per cent of the cases, at least one of the parents gave a history of having been enuretic, and in 35 per cent of the cases at least one additional sibling was enuretic.

Only 20 per cent of those receiving the drug evidenced transitory drowsiness during the early phase of treatment. Although lack of drowsiness does not prove the tranquilizer ineffective, one may question the adequacy of the dose of the drug.

The role of "giving medicine" to these children could not be isolated from the total treatment plan. This, of course, could have been evaluated should there have been a

Table III

Designated level of improvement	*No. of cases*	*% of total cases treated for 3 months*		
Cured	9	14	45	73
Greatly improved	21	31		
Improved	19	28		
Unchanged	18	27		
Total	67	100		

third group in the study not receiving a drug or placebo.

A follow-up study will be undertaken to ascertain whether or not the progress has been maintained and if some "breaking up" of the chronic pattern leads to further improvement with time.

SUMMARY

One hundred enuretic children, aged 5 to 14 years, were placed after evaluation in two groups, one receiving meprobamate, the other a placebo, both receiving supportive and suggestive management over a 3-month period.

There was no statistically significant difference in improvement between the drug and the placebo groups, but the total treatment plan effected cure or great improvement in 45 per cent of the children. Improvement was greater the younger the child.

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Cryptococcus meningitis in a child successfully treated with amphotericin B, with a review of the pediatric literature

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CRYPTOCOCCOSIS, also known as torulosis or European blastomycosis, is a mycotic disease of man and animals caused by *Cryptococcus neoformans (Torula histolytica* or *Cryptococcus hominis)*. The first case reported was by Zenker[1] in 1861 in a man dying from a fungous infection involving the central nervous system, and it is most probable that the organism was *C. neoformans*. Büsse[2] and Buschke[3] independently recovered yeastlike organisms from a woman with bone lesions who died from generalized involvement. The organisms were referred to as *Saccharomyces neoformans*. Vuillemin[4] recognized the lack of ascospores, thus differentiating it from true yeast, and called it *C. hominis*. The generic term *Cryptococcus* was used or given by Kützing.[5] In 1902, Frothingham[6] recovered the organism from a pulmonary lesion in a horse, and Von Hansemann[7] in 1905 recovered the organism for the first time from a man dying of meningitis. In 1916, Stoddard and Cutler[8] recorded the clinical and pathologic features of the disease, described the morphologic and cultural characteristics of *C. neoformans*, and proved it to be a true yeast, reproduced by budding only. Because it fails to produce mycelium or ascospores, it is now classified with the anascosporogenous yeasts.

In Lodder's[9] classification *Cryptococcus* has 25 species, all saprophytic except *C. neoformans*. Saprophytic strains are widespread in nature,[10] and are found in soil,[11] food, and the skin and gastrointestinal tract of man[12] and animals.[10] Pathogenic strains have been recovered from peaches,[13] milk, and from the oropharynx, gastrointestinal and vaginal tracts of apparently healthy carriers.[14] Some relation seems to exist to pigeon manure.[10]

The yeasts are thought to enter the body

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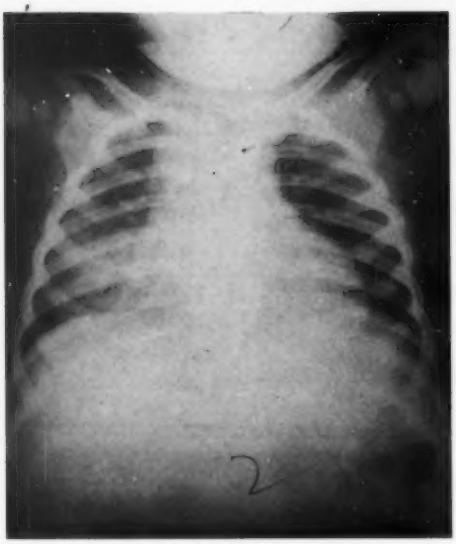

Fig. 1. Right middle lobe infiltrate seen on admitting film.

through the respiratory tract, which explains the frequency of lung involvement,[14] although the tonsils and skin are considered the portal of entry by Freeman.[15]

Cryptococcosis is world wide in distribution but the largest number of cases has been reported in the United States, where approximately 450 cases have been recorded in adults. The actual incidence of the disease cannot be estimated accurately by the number of published cases.

Alteration of the host-parasite relationship, disturbance in the reticuloendothelial system, and debilitating disease contribute to the lowering of resistance to the organisms. Coexistence of cryptococcosis with Hodgkin's disease,[20-23, 27, 28] leukemia, reticulum cell sarcoma,[25, 27] histoplasmosis,[28] tuberculosis,[28] moniliasis,[25] and diabetes[24] have been previously described.

The organism most likely reaches the central nervous system by way of the blood stream,[16] producing 3 varied types of pathologic pictures: (1) meningitis, where the subarachnoid space of the base of the brain is most frequently involved; (2) meningoencephalitis, where the gray matter is mostly affected with a honeycomb appearance of the cortex, basal ganglia, brain stem, thalamus, or cerebellum; and (3) cryptococcal granuloma, which is frequently found in the cerebellum and cerebrum. Other organs such as the lung, liver, spleen, skin, and lymph nodes are less frequently involved.[14] In 1937 Levin[17] reviewed the literature and since then numerous other reviews have been published,[14, 18, 19] but none in the pediatric literature. The prognosis, except for the localized, cutaneous type, is grave, especially when the central nervous system is involved. Until the advent of amphotericin B,* these cases were nearly always fatal.

We have recently encountered in a child a case of *C. neoformans meningitis* associated with tuberculosis of the lung. To our knowledge, this is the first child with cryptococcal meningitis to have apparently recovered completely when treated with amphotericin B. Certain aspects of this case will be discussed and the review of the pediatric literature on cryptococcal meningitis is included.

CASE REPORT

G. H.,† an 18-month-old Negro boy, was brought in a comatose condition to the emergency room of the Mount Sinai Hospital, Chicago, on Aug. 21, 1960, with tonic and clonic convulsions and a temperature of 104° F. For about one month prior to admission, the child had had frequent colds, a cough, and fever but improved when given penicillin by a local physician. Three days prior to admission, the child again developed a dry cough, rhinorrhea, and fever. On the day of admission, shortness of breath was also noted.

In the emergency room, the child was comatose; the pupils were equal, dilated, and unresponsive to light. There was a slight ptosis of the left eyelid, the neck was supple,

*The trade-mark of E. R. Squibb & Sons for amphotericin B is Fungizone.

†From the service of Dr. H. S. Gordon.

and Kernig's, Brudzinski's, and Babinski's signs could not be elicited. The throat was slightly reddened, the ears clear, and no significant lymphadenopathy could be found. The breath sounds were harsh, but with no râles or dullness to percussion. The heart was normal, but there was a tachycardia. The abdomen was soft, and no organs or masses could be felt.

Phenobarbital, intramuscularly, and aspirin suppositories were given, but convulsions continued for another half hour.

A roentgenogram of the chest was immediately taken and a moderate pneumonia of the right middle lobe of the lung was visualized (Fig. 1).

The fundi showed no papilledema, and a lumbar puncture was performed. The pressure was slightly elevated, and the clear fluid obtained included 217 cells per cubic millimeter, predominantly neutrophils. The protein was elevated, and the Pandy test was positive. Glucose and chloride concentrations were normal. Organisms resembling Cryptococcus were seen in this specimen, but, unfortunately, no cultures were made on it.

Examination of the blood showed a hemoglobin of 8.1 Gm. per 100 ml., hematocrit 28, red blood cells 3.75 million per cubic millimeter, and color index 0.69. The leukocyte count was 8,100 per cubic millimeter with 3 per cent bands, 55 per cent neutrophils, 34 per cent lymphocytes, 5 per cent eosinophils, 2 per cent monocytes, and 1 per cent basophils. The sedimentation rate was 30 mm. per hour.* The blood urea nitrogen was 18.5 mg. per cent.

The child was given 1,000,000 units of penicillin, intravenously, twice a day; 250 mg. sulfadiazine, intravenously, every 6 hours; and 50 mg. per kilogram chloramphenicol, intramuscularly, every 6 hours. On the second hospital day, his neck become slightly rigid. A Mantoux test (1:10,000 OT.) was markedly positive after 24 hours. The child was then immediately placed on para-aminosalicylic acid, 750 mg., 3 times a day; isoniazid, 100 mg., 3 times a day; streptomycin, 50 mg., intramuscularly, twice a day; and prednisone, 10 mg., twice a day.

One the third hospital day, the patient was alert and free of convulsions. The lumbar puncture was repeated, the cell count was 207 per cubic millimeter; 90 per cent of them, however, were lymphocytes in this specimen. India ink preparations showed numerous encapsulated cells morphologically typical of *C. neoformans*. A direct count of the organisms showed them to be greater than 10,000 per cubic millimeter. A diagnosis of cryptococcal meningitis was made and amphotericin B, 15 mg. given intravenously in 500 ml. of dextrose in water with addition of 0.5 ml. heparin as a precaution against thrombophlebitis, was added to the therapeutic regimen. Potassium iodide was also given, starting at 3 drops twice a day and increased by 1 drop per day until a dose of 12 drops twice a day was reached. This was stopped on the sixty-second day of hospitalization when the lung lesion was felt to be a tuberculous rather than a cryptococcal granuloma. The history from the child's parents revealed that the patient was in close contact with an uncle who had been treated for tuberculosis since February, 1960. It was also learned that the child had been playing around pigeon excreta.

The course of the disease and the treatment are shown in Fig. 2. The prednisone, chloramphenicol, and penicillin were discontinued after the third hospital day. The amphotericin B was given every other day or 3 times weekly when the blood urea nitrogen became abnormally elevated. This dosage was found to be best. During the last 2 weeks of therapy, amphotericin B was given once a week in a dose of 30 mg. Despite intensive drug therapy, liver function tests remained normal, and the drug was well tolerated.

Throughout the long hospital course, every effort was made to isolate the tubercle bacillus from gastric washings, spinal fluids, and bronchial aspirations. All smears, cultures, and guinea pig inoculations were negative for acid-fast bacilli. Skin and serologic tests

*Wintrobe method.

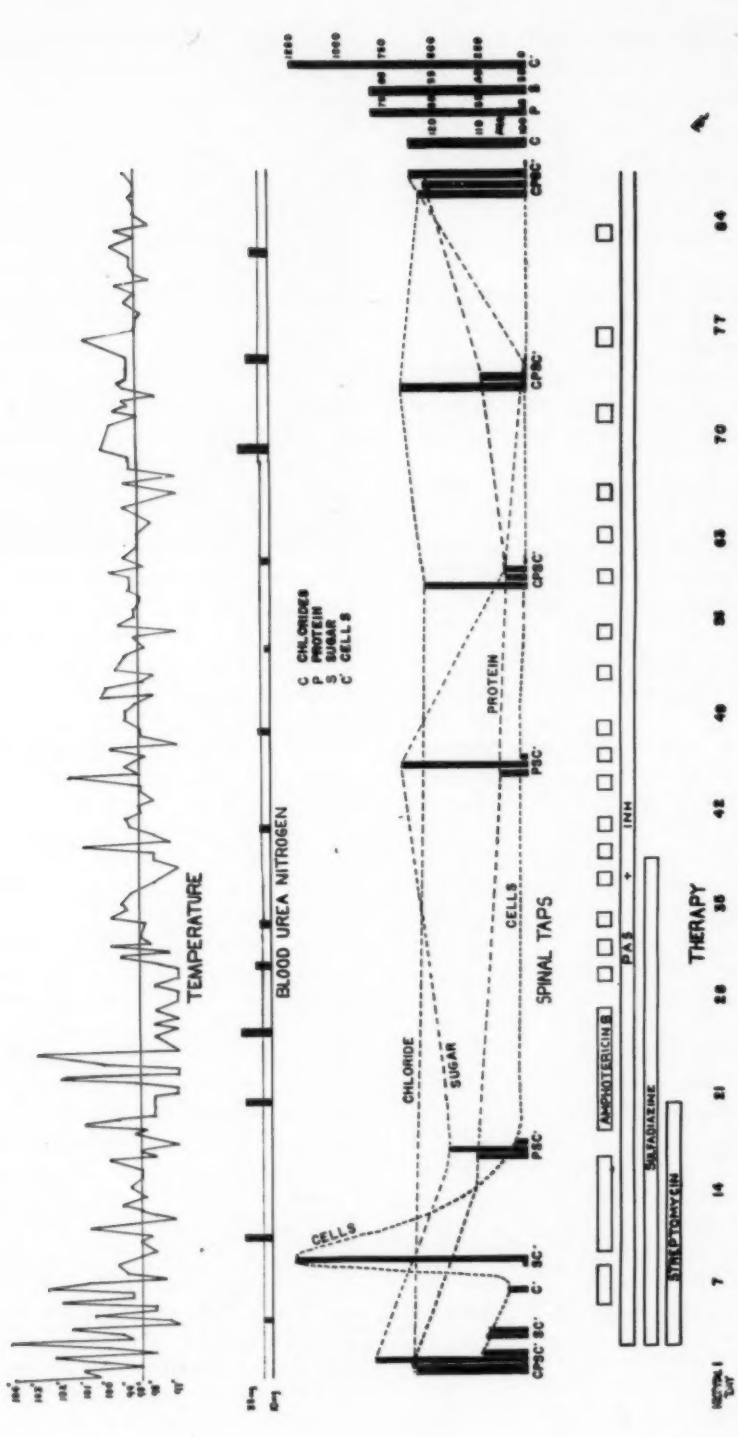

Fig. 2. Clinical course, therapy, and laboratory findings.

for blastomycosis, coccidioidomycosis, and histoplasmosis were all negative. The cryptococci were seen in large numbers in the first 5 specimens of spinal fluid, but none in the succeeding 4 specimens. The number of organisms, the size of the capsule, and the amount of budding diminished strikingly with each succeeding specimen. Every effort to culture the organism, however, failed. All animal inoculations were also negative. It is probable that the intensive therapy with the antibiotics and amphotericin B were responsible for the negative cultures. Ordinarily, this organism grows with ease on ordinary laboratory media, either at room or incubator temperature.[12, 14]

The child progressively improved during his hospitalization, although the lung lesion did not diminish in size (Figs. 3, 4, and 5). The child was discharged from the hospital after the spinal fluid findings returned to normal on his eighty-seventh hospital day. He was continued, however, on antituberculosis therapy.

Five months after discharge, the child has remained in good health, is gaining weight, and is mentally normal, with a normal electroencephalogram. The pulmonary lesion has decreased in size on the continued antituberculosis therapy (Figs. 6*A* and 6*B*).

REVIEW OF THE LITERATURE

To date, including the report of the present case, we have been able to find only 23 cases of cryptococcus meningitis in children less than 15 years of age. Table I summarizes the significant data on these cases.

The ages of the patients ranged from 20 minutes post partum to 15 years of age. The presence of the disease in a 20-minute-old newborn[48] is definite evidence of the existence of a congenital cryptococcal infection. Of the 23 cases, 3 definite cases and 3 equivocal cases appeared in the neonatal period.

The sex distribution, as in the adults, favors the males, although such a distribution is difficult to explain. The disease is not limited to the white race; 5 of the patients were Negroes. The brain and the meninges were involved in 23 cases, the lungs in 9, the liver in 8, the spleen in 7, the kidney in 5, the eyes in 4, the skin in 3, the bones, adrenals, and thyroid in 2, and the pancreas, heart, and lymph nodes in 1.

In the pediatric age group, cryptococcosis is also frequently associated with other diseases such as histoplasmosis,[39] moniliasis,[43] Hodgkin's disease,[37] sarcoidosis,[46] and tuberculosis.[49]

Cryptococcosis is a subacute or chronic

Fig. 3. Persisting lung lesion, 1 month later.

Fig. 4. Persistance of lung lesion, 2 months later.

Table I. Review of the world pediatric literature

Author	*Country*	*Year*	*Age, sex, race*	*Duration of symptoms before admission*	*Physical findings and presenting symptoms*	*Cerebrospinal findings*
Barlow[29]	Australia	1923	3, F, W	4 weeks	Diarrhea, irritability, drowsiness, and signs of meningeal irritation	No definite number cells, all lymphocytes ↑Pressure
Carton and Mount[30]	United States	1933	12, F, W	6 weeks	Headache, vomiting, poor vision, hyporeflexia, Babinski and Kernig +, left ptosis, sixth nerve palsy, papilledema, retinal hemorrhage and exudation, coma, and death	88 cells, 90% lymphocytes ↑Pressure Protein 100 Glucose 40
Reeves, Butt, and Hammack[31]	India	1938	15, F, W	9 months	Headache, vomiting, stiff neck, blurred vision, cough, fever, emaciation, and optic atrophy	97 cells, mostly polymorphonuclear leukocytes, lymphocytes later ↑Pressure at first, normal later Protein↑
Longmire and Goodwin[32]	United States	1939	4, F, W	3 months	Fever, dyspnea, convulsions, stupor, deafness and blindness, emaciation, cyanosis, stiff neck, and papilledema	100 to 400 cells, polymorphonuclear leukocytes 75, lymphocytes 25 ↑Pressure Protein↑ Glucose 35
D'Aunoy and Lafferty[33]	United States	1939	7, M, N	Sudden	Stiff neck, semicoma, and acute illness	100 to 250 cells ↑Pressure
Marshall and Teed[34]	United States	1942	9, F, W	6 weeks	Fever, headache, nausea, irritability, vomiting, much rigidity, Kernig +, papilledema, and retinal hemorrhage	8 to 15 cells ↑Pressure
Dormer, et al.[35]	South Africa	1945	12, M, N	4 months	Cough, headaches, anorexia, weight loss, clubbing of fingers, fever, and dullness in right upper chest	Many polymorphonuclear leukocytes
Hamilton and Thompson[36]	United States	1946	6, M, N	2 weeks	Malaise, anorexia, fever, headache, vomiting, generalized convulsion, papilledema, stiff neck, Brudzinski +, and hyporeflexia	320 cells, mostly polymorphonuclear leukocytes ↑Pressure Protein 180 Glucose > 10
Debre, et al.[37]	France	1946	12, M, W	Unknown	Headache, vomiting, fever, convulsions, photophobia, opisthotonos, papilledema, hepatosplenomegaly, ulcer on neck, and mass over clavicle	Not done

Direct smear of cryptococcus	Culture	Ante-mortem diagnosis	Duration of illness	Organs involved	Autopsy	Treatment	Remarks
Positive from CSF	–	Yes	3 months	Brain	Yes	None	Optic atrophy prior to death
Positive from CSF	Positive from CSF	Yes	5 weeks	Brain	No	KI orally, NaI intravenously, 5% NaI, and acriflavine intrathecally	Skull x-ray showed signs of increased intracranial pressure
Positive from CSF and chest abscess aspiration	Positive chest abscess aspiration	Yes	2 years	Brain	–	Sulfadiazine Torula antigen, KI	Skull x-ray—increased marking Chest x-ray—interlobar empyema CSF still positive 2 years later Reported to be well 9 years later, but with sequelae
Positive from CSF	Positive from CSF	Yes	3 months	Brain, meninges, lung, thyroid, liver spleen, and kidney	Yes	–	–
Positive from CSF	Positive from CSF	No	48 hours	Brain	Yes	–	–
Positive from CSF	Positive from CSF	Yes	5 months	Brain	–	Sulfadiazine	Living, well, subnormal intelligence 4 years later
Positive from CSF	Positive in guinea pig	Yes	5 months	Lung and brain	–	Sulfadiazine, KI	Postoperative meningitis following right upper pulmonary lobectomy Patient survived, no follow-up
Positive from CSF Positive from sputum	Positive from cisternal puncture; positive from sputum	Yes	72 days	Lungs	–	Sulfadiazine	Chest x-ray—nonspecific lung infiltration Pneumoencephalogram—enlargement of one ventricle and other not visualized
–	Positive from CSF	Yes	2 weeks	Skin, lungs, heart, spleen, liver, and brain	Yes	KI	Coexisting Hodgkin's disease

Table I. Cont'd

Author	*Country*	*Year*	*Age, sex, race*	*Duration of symptoms before admission*	*Physical findings and presenting symptoms*	*Cerebrospinal findings*
Cox and Tolhurst[38]	Australia	1946	7, M, W	5 weeks	Headache, vomiting, leg pain, semicoma, active deep reflex, paralysis of right leg and arm, stiff neck, and papilledema	41 to 50 cells, 40 to 46% lymphocytes ↑Pressure Protein↑
Mider, Smith, and Bray[39]	United States	1947	12, M, N	3 months	Weakness, weight loss, anorexia, fever, and red swollen sternoclavicular region which drained, generalized lymphadenopathy	–
Greening and Menville[40]	United States	1947	6, M, N	2 weeks	Upper respiratory infection, headache, vomiting, convulsion, and stiff neck	300 cells, lymphocytes 30% ↑Pressure ++Pandy Protein↑ Glucose, normal
Cloward[41]	Hawaii	1948	5½, M, Chinese	1 week	Septic temperature, headache, irritability, convulsions, paralysis, retinal hemorrhages, and papilledema	450 cells, lymphocytes 33% ↑Pressure Protein 114 to 140
Neuhauser and Tucker*[42]	United States	1948	7 weeks, M, W	7 weeks	Vomiting, twitching, rigidity, hydrocephalus, cataract and chorioretinitis, ankle clonus, and Babinski +	27 to 36 cells, 75% lymphocytes Protein 1,400 Glucose 69
Neuhauser and Tucker*[42]	United States	1948	19 days, M, W	19 days	Jaundice since birth, hepatosplenomegaly, dark urine and clay stool, hydrocephalus, and malnourishment	–
Neuhauser and Tucker*[42]	United States	1948	17 days, M, W	4 days	Irritability, convulsions, hypertonicity, frequent clonic motions of extremities, hepatosplenomegaly and chorioretinitis	–
Spicer, Hiatt, and Kessel[43]	United States	1948	8, M, W	4 years	Headache and lethargy, repeated episodes of pulmonary infiltration with chronic cough, and septic temperature	Cloudy ↑Pressure
Nassau and Weinberg-Heiruti[44]	Israel	1948	12 days, M, W	5 days	Cyanosis and generalized convulsion, no nuchal rigidity, hyporeflexia, Kernig +, splenomegaly, skin macular and papular lesions, and hydrocephalus	5,000, later 263 cells Protein 238 to 240 Glucose 14 mg.
Health[45]	United States	1950	27 days, M, W	–	Endophthalmitis with massive separation of retina	–
Lepau, et al.[46]	United States	1953	10, F, N	3 months	Anorexia, weakness and generalized lymphadenopathy, later headache, fever, vomiting, coma, and death	2,720 cells, 95% polymorphonuclear leukocytes, later 16,000 cells Protein 70 to 200 Glucose 10 to 20 Cl 110 to 121

Direct smear of cryptococcus	*Culture*	*Ante-mortem diagnosis*	*Duration of illness*	*Organs involved*	*Autopsy*	*Treatment*	*Remarks*
Positive from CSF	Positive from CSF	Yes	2½ months	Lungs and brain	Yes	–	Chest x-ray—fibrosis and small nodules
Positive from blood and bone marrow	–	No	11 days	Brain, liver, kidney, and bones	Yes	–	Associated with generalized histoplasmosis
–	Positive from CSF	Yes	2 months	Brain	No	KI, sulfadiazine, and penicillin intrathecally	Died
Positive from CSF	Positive from CSF	Yes	28 days	Brain	Yes	Penicillin, sulfadiazine	Pneumoencephalogram performed Exploratory trephination for brain abscess—negative
–	–	No	23 days	Brain, eyes, and lungs	Yes	–	Skull x-ray—calcification of frontal region Punctate and calcification along ventricles
–	Positive post-mortem from CSF	No	4 days	Spleen, liver, brain, and bone	Yes	–	Skull x-ray—punctate calcification of the brain
–	Positive post-mortem from CSF	No	10 days	Brain, liver, spleen, kidney, lungs, and eyes	Yes	–	Skull x-ray—calcifications of cortex and brain substance
–	Positive from CSF and urine	No	35 days	Brain, lungs, kidney, spleen, pancreas, adrenal, and liver	Yes	Sulfadiazine	Associated with generalized candidiasis
Positive from CSF	Positive from CSF after death	No	6 weeks	Brain and skin	Yes	Penicillin, sulfadiazine	–
–	Positive from CSF	No	27 days	Many organs including eyes and brain	Yes	–	Congenital origin Combined lesion with toxoplasmosis
–	Positive from CSF, nose and throat	Yes	61 days	Brain	Yes	Polymyxin B, intramuscularly, and nystatin intrathecally	Coexisting Boeck's sarcoid

Table I. Cont'd

Author	*Country*	*Year*	*Age, sex, race*	*Duration of symptoms before admission*	*Physical findings and presenting symptoms*	*Cerebrospinal findings*
Soysal, Unat, and Tahsinglu[47]	Turkey	1954	7, M, W	3 months	Ulcerative crusting skin lesions, acute illness, cough, fever, lymphadenopathy, membranous tonsils, papilledema, and hepatosplenomegaly	–
Oliviera Campos[48]	Portugal	1955	32 weeks, premature, F, W	At birth	Cyanosis, brachycardia, weak respiration, anasarca, hydrocephalus, and hepatosplenomegaly	–
Nanda, et al.[49]	United States	1956	10, M, W	3 weeks	Headache, nausea and vomiting, emaciation, lethargy and weakness, bilateral papilledema and nuchal rigidity, ankle clonus, and Babinski +	84 cells, 80% lymphocytes ↑Pressure

*Personal communication with the authors indicates that the 3 cases quoted had toxoplasmosis and the cryptococcosis was not

infection. Cases with pulmonary involvement are usually asymptomatic or may have a slight cough, scanty mucoid sputum, and infrequent hemoptysis. There is rarely fever, malaise, and weight loss. Clinical symptoms and physical signs become evident when meningitis supervenes. Fever, headache, vertigo, nausea, irritability, vomiting, stupor, confusion, lethargy, and convulsions are noted. Nuchal rigidity, positive Brudzinski's and Kernig's signs, as seen in other meningitides, appear. Later, increasing intracranial pressure develops with its associated signs of papilledema, ophthalmoplegia, and cranial nerve involvement. Fever is seldom above 101° F., the leukocyte count is variable, and the erythrocyte sedimentation rate is usually normal. The spinal fluid is under increased pressure. It may be clear but is generally turbid, and, on rare occasions, xanthochromic. The cell count is usually between 40 and 1,000 cells with an average of 200 to 300 per cubic millimeter[3]; the cells are mostly lymphocytes, although at an early stage they may be polymorphonuclear leukocytes. Total protein is increased and glucose and chlorides are slightly decreased. In the neonatal period, cryptococcosis presents itself with different symptoms than in older children. Cyanosis, convulsions, failure to thrive, jaundice, hepatosplenomegaly, chorioretinitis, skin rash, and punctate calcifications in the brain may be noted. Thus cryptococcal meningitis must be differentiated from other diseases, especially congenital toxoplasmosis and cytomegalic inclusion disease.[56-58] Cryptococcosis, or other fungous invaders of the central nervous system, should be considered in the differential diagnosis of meningitis or expanding intracranial lesions. This will be frequently suggested by pleocytosis of the cerebrospinal fluid, reduced glucose and chloride levels, and increase in protein and pressure. Specimens of fluid should be cultured for fungi on Sabouraud's and other media and India ink preparations should be examined.

The pathogenicity of strains of *C. neoformans* is determined by laboratory methods such as those described by Benham.[12] The

Direct smear of cryptococcus	Culture	Antimortem diagnosis	Duration of illness	Organs involved	Autopsy	Treatment	Remarks
–	–	No	10 days	Brain, skin, lymph nodes, thyroid, tonsils, liver, spleen, kidney, adrenal, and eyes	Yes	Penicillin and sulfadiazine	Chest x-ray—normal
–	–	No	20 minutes	Brain, liver, and spleen	Yes	–	Lived for 20 minutes
Positive from CSF	Positive from CSF	Yes	6 months	Brain	–	Ethyl vanillate	Coexisting tuberculous meningitis Living with residual spasticity of left hand and wrist, and hyperkinetic behavior EEG—diffuse organic dysrhythmia

definitely proved.

characteristic features are fermentation of all sugars, except lactose, good growth on most culture media, and pathogenicity to mice. Staining of the capsule with mucicarmine and the appearance in India ink preparations are pathognomonic of this organism.[14]

Treatment of cryptococcosis was a challenge until amphotericin B was introduced. Previous treatment consisted of roentgen therapy,[40] sulfonamides,[34, 51] diamidines,[52] iodides,[14] penicillin, tetracycline, ethyl vanillate,[49, 54] nystatin,[53] and cycloheximide.[55] The prognosis was poor and most pediatric patients died. Out of the group reviewed, only 5 survived, but with neurological sequelae in 3; 2 had no follow-up.

DISCUSSION

There are three interesting features in our case: the association of tuberculosis and cryptococcosis; the failure to culture the fungus despite the numerous organisms seen; and the uneventful recovery with amphotericin B.

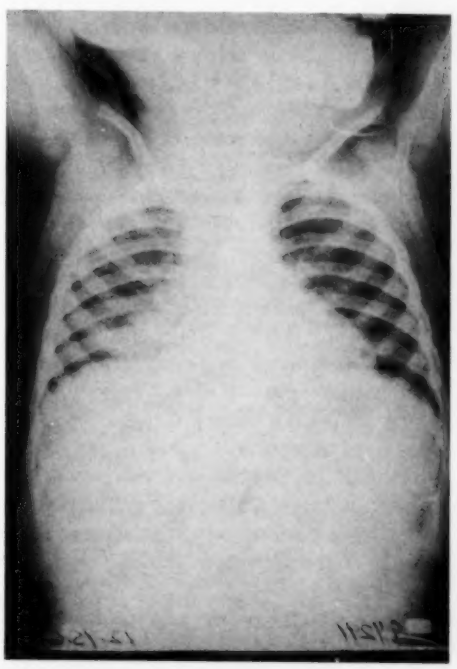

Fig. 5. Lung lesion still present 3 months later.

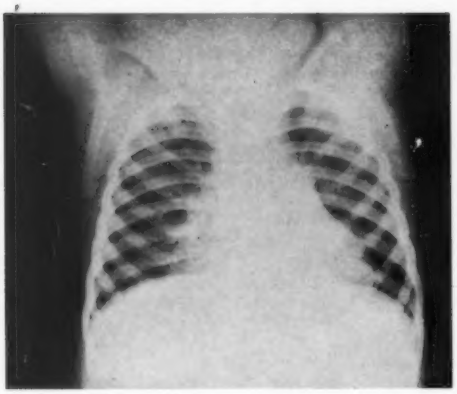

Fig. 6*A*. Lung lesion decreasing in size on antituberculosis therapy, 5 months later.

The diagnosis of pulmonary tuberculosis in the child was supported by the history of contact with the disease, the positive tuberculin test, and the resolution of the persisting pulmonary lesion during prolonged antituberculosis therapy. Additional evidence for the diagnosis was the location of the lung lesion. In cryptococcosis, the lesion is twice as common in the lower lobe as in the upper and middle lobes. In 50 per cent of reported cases, there is involvement of 2 or more lobes and in about one third there is diffuse involvement of both lungs.[59, 60-62] This is in contrast to our case, where the lesion was in the right middle lobe.

The failure to recover tubercle bacilli on culture and animal inoculation could be attributed to the intensive antituberculous therapy instituted prior to bacteriologic examination.

The failure to culture Cryptococcus despite the numerous organisms repeatedly seen on smear, may likewise be due to the intense antibiotic and amphotericin B therapy. On administration of the drug, the capsule of the fungus decreased very strikingly in size.

The uneventful recovery from this usually fatal disease, we feel, is due to the very intense therapy with the antifungal antibiotic amphotericin B.

It is recommended that therapy with amphotericin B be started with 0.25 mg. per kilogram daily and that the dose be gradually increased until an optimum level is attained; this is generally achieved with 1.0 mg. per kilogram. A dose exceeding 1.5 mg. per kilogram is cautioned against. We instituted therapy with a dose of 1.25 mg. per kilogram and maintained this dose despite the azotemia. There were rare occasions when symptoms of nausea, vomiting, and irritability were manifested. When the drug was administered every other day or 3 times weekly, the azotemia disappeared. During the last 3 weeks of hospitalization the dose was doubled to 2.5 mg. per kilogram, but was given only once a week. This did cause, once again, an elevation of the blood urea nitrogen but was otherwise well tolerated. The patient received a total of 490 mg. of the drug during a 12-week course. Liver function studies were within normal limits.

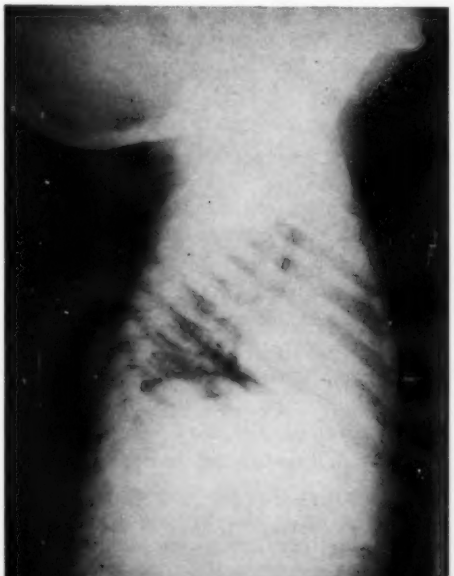

Fig. 6*B*. Lateral film showing regression of middle lobe lesion.

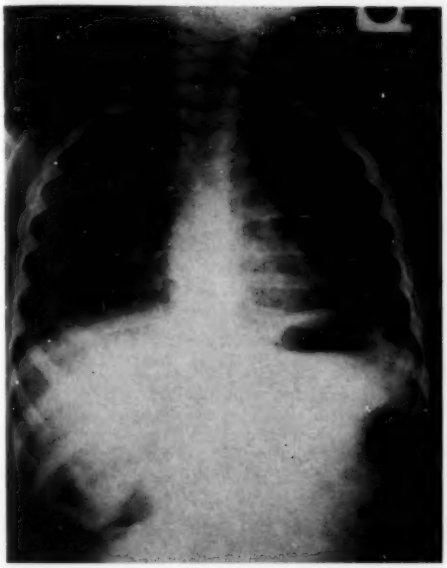

Fig. 7. X-ray 7 months after patient was discharged from the hospital. The chest x-ray revealed further resolution of the right middle lobe lesion. The patient is still being maintained on antituberculosis therapy as indicated in our paper.

Another side effect of the drug is anemia. This may have been the cause of the slight drop noted in our patient's hemoglobin level despite supplemental iron therapy, but this was only transient and soon returned to its previous level.

SUMMARY

A case of cryptococcal meningitis associated with pulmonary tuberculosis is reported.

The treatment of the disease with amphotericin B is discussed; the drug was effective and well tolerated.

A review of cryptococcosis in the pediatric age group from the world literature is presented and the differences in the clinical manifestations of the disease in the neonate and the older child are stressed. Evidence is presented for the occurrence of congenital cryptococcal meningitis. In the pediatric age group, patients with histoplasmosis, moniliasis, Hodgkin's disease, leukemia, sarcoidosis, and tuberculosis manifesting meningeal symptoms should be watched for superimposed cryptococcal infection. The almost 100 per cent mortality rate of the 23 cases reviewed before the use of amphotericin B stresses the paramount role the drug plays in the management and treatment of this fungal disease.

ADDENDUM

Since this article was submitted for publication a paper has appeared in the *British Medical Journal* (vol. 2, pp. 91-93, 1961) entitled "Cryptococcal Meningo-Encephalitis," by W. F. Twining McMath and K. K. Hussain. This article deals with a case of cryptococcal meningoencephalitis in a 4-year-old boy. The child was treated with steroid therapy, 5 mg. doses of prednisolone, tetracycline, 250 mg. every 6 hours, amphotericin B, 0.5 mg. in 5 ml. of water intrathecally and 20 mg. in 250 ml. of 5 per cent dextrose by intravenous drip. He died 3 days following institution of therapy.

We wish to thank Dr. H. S. Gordon and the pediatric staff, and the nursing staff, headed by Miss D. Melchior, at Mount Sinai Hospital, Chicago, Ill., for their help.

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Adrenal cyst

A case report and a review of the pediatric literature

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CHICAGO, ILL.

CYSTS of the adrenal gland are rare in the pediatric age group. Most of those recorded in the medical literature have been small lesions discovered at autopsy.

We have encountered a large adrenal cyst in a newborn infant and present here the clinical manifestations, the pathologic description, and a review of the pediatric literature relative to cysts of the adrenal gland.

CASE REPORT

B. B., a 10 pound, 11 ounce, white male infant was born on March 10, 1955, following a normal gestation and uneventful delivery. On routine examination at birth an abdominal mass was palpated; he was sent to the Mount Sinai Hospital of Chicago for evaluation.

The physical examination revealed a pale but active infant who had a 10 cm. by 6 cm. mass in the abdomen. The mass was irregular, lobulated, and filled the entire left hypochondrium and lumbar areas, and about one third of the left lower quadrant of the abdomin where a bulge was visible. The bowel could be palpated anterior to the mass, but was displaced toward the midline. The left kidney could not be delineated, while the right was normally palpable; the liver and spleen could not be felt. Large hydroceles were present bilaterally.

The initial laboratory examination revealed: a red blood cell count of 3.9 million per cubic millimeter; hemoglobin, 12.8 Gm. per 100 ml.; a leukocyte count of 14,900 per cubic millimeter, of which 32 per cent were segmented cells, 59 per cent were lymphocytes, 3 per cent eosinophils, and 6 per cent monocytes. A moderate number of macrocytes were noted. The serum sodium concentration was 141 mEq.; the chloride, 108 mEq., and potassium, 4.2 mEq. per liter; urea nitrogen, 14 mg. per 100 ml.; and the total serum bilirubin was 0.8 mg. per 100 ml. The infant's blood type was the same as the mother's, AB, Rh positive.

A roentgenogram of the abdomen revealed a mass in the left side which displaced the gas-filled intestine medially (Fig. 1). In the lateral view, the mass appeared to be retro-

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peritoneal. An intravenous pyelogram was attempted but revealed only poor excretion of the dye.

On the third day of life the hemoglobin count decreased to 9.8 Gm. per 100 ml. and the red blood cell count to 2.98 million per cubic millimeter. The white blood cell count was 12,000 per cubic millimeter with 2 per cent metamyelocytes, 21 per cent stab forms, and 49 per cent segmented cells. The red blood cells were 1.2 per cent reticulocytes, and the color index was 1.06.

A laparotomy was performed, and, through a transabdominal incision, the mass was exposed and found to be an 11 by 6.5 by 5 cm. multilocular cystic tumor arising from the lower pole of the left adrenal gland. The left kidney was normal, but was displaced inferiorly by the suprarenal mass. The tumor and the adrenal gland were removed. Hemostasis during the operation was excellent and the blood loss was minimal. The infant withstood the procedure well. He received 150 c.c. of whole blood during the operation.

Five hours post operative the patient developed acute respiratory distress with dyspnea, intercostal retractions, and a respiratory rate of 55 per minute. Coarse râles and ronchi were present throughout both lung fields. Bronchoscopic aspiration removed moderate amounts of frothy mucus from the trachea and major bronchi, and there was moderate edema of the epiglottis. A roentgenogram of the chest revealed no evidence of cardiac or pulmonary abnormality. Penicillin and tetracycline were then prescribed.

Following the immediate postoperative respiratory distress, the infant made an uneventful recovery. Despite the apparent clinical improvement, the infant's hemoglobin failed to return to the anticipated normal values. Immediately after the operation the hemoglobin was 10.7 Gm. per 100 ml., and the red blood cell count was 3.6 million per cubic millimeter; the color index was 0.96, and the total eosinophilic count was 72 per cubic millimeter. Two days later the hemoglobin dropped to 9.2 Gm. per 100 ml., the red blood cell count to 2.8 million per cubic millimeter, but the reticulocyte count remained at 1.2 per cent. Studies to determine the cause of the anemia were undertaken since no evidence of postoperative bleeding could be found. The total bilirubin was 0.8 mg. per 100 ml., the direct and indirect Coombs tests were negative, and the red blood cell fragility test was normal. A second blood transfusion of 100 c.c. of whole blood was given and the hemoglobin rose to 12.5 Gm. per 100 ml., and the red blood cell count to 4.1 million per cubic millimeter. The total eosinophilic count on the 7th postoperative day was 688 per cubic millimeter. Eight days later a bone marrow study was done and showed depression of the erythrocytes. The ratio of myeloid to erythroid cells was 7 to 1 (normal for this age, 5 to 1). From the beginning of the second week the baby's anemic condition improved. When discharged

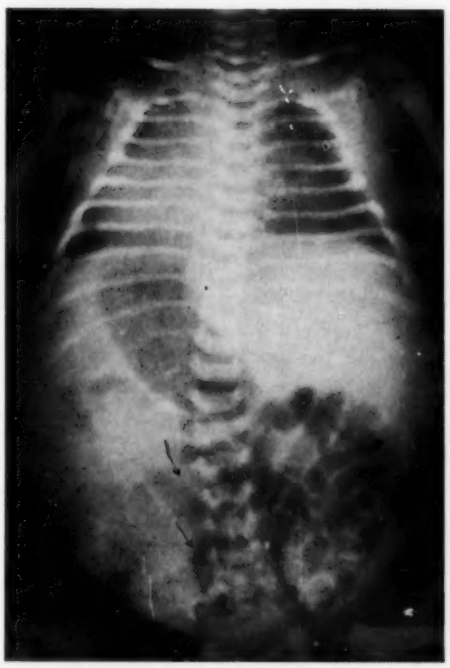

Fig. 1. X-ray of the abdomen showing abdominal enlargement and displacement of the bowel to the right by the radiopaque mass occupying the whole of the left abdominal cavity.

Table I. Adrenal cyst. Review of the pediatric literature

Author	*Year*	*Age*	*Sex*	*No. cases*	*Symptoms*	*Side*	*Size*	*Diagnosis by*	*Type of fluid*	*Calcification*	*Type of cyst*	*Miscellaneous*
Belaieff[9]	1886	2 Months	M	1	Gray pigmentation of skin, convulsion, Coma	Bilateral	⅔ of adrenal gland; large adrenal gland	Autopsy	Clear	No	Lymphangiectasia, without good description of tumor	Addison's disease secondary to cyst
Schmieden and Peiper[10]	1926	5½ Months	F	1	Abdominal distention and pyodermia	Left	6 by 5 by 5 cm.	Pneumoperitoneum, operation	Blood	No	Pseudocyst in hemangioendothelioma	Birth trauma thought to be the cause of bleeding into previously present tumor; patient survived; no endocrinopathy
Campbell[11]	1937	Below 5 Years	?	7	None recorded	4 Right 1 Left 2 Bilateral	0.5 to 1.5 cm.	Autopsy	Clear	?	?	No endocrinopathy
Moore and Cermak[12]	1950	8 Months	M	1	Pigmentation of skin, vomiting, diarrhea, acidosis	Bilateral	4.5 by 2.5 by 1.5 cm.	Autopsy	Brown, bloody	No	Pseudocyst	Addison's disease secondary to cyst
Abeshouse, Goldstein, and Abeshouse[18]	1959	3 Years	M	1	Pain right loin, vomiting, weight loss, fatigue, noticeable mass	Right	?	Palpation, and operation	Blood	Yes	Pseudocyst	Flat film: calcium below right kidney, retrograde displacement of kidney
Our case	1960	Newborn	M	1	Mass on left side, anemia	Left	11 by 6.5 by 5 cm.	Operation	Blood	No	Hamartoma	No endocrinopathy; hemorrhage within tumor mass; patient survived

after 14 days of hospitalization he weighed 2½ ounces above his birth weight.

During the past 5 years the child's growth and development have been normal. At the age of 5 years, the 17-ketosteroids were 1.2 mg. per 24 hours, and the urinary 17-hydroxycorticoids (Glenn-Nelson method) were 1.4 mg. per 24 hours, both within normal limits.

PATHOLOGIC OBSERVATIONS*

The surgical specimen consisted of a multilocular cystic mass which measured 11 cm. by 6.5 cm. by 5.5 cm. (Fig. 2A), the external surface was smooth and glistening, and the mass was tense to palpation. On the cut section there were numerous cysts ranging from 0.5 cm. to 5.5 cm. in diameter. Some were filled with hemorrhagic fluid, others with necrotic-appearing material, and some contained a gray, jellylike material. The walls of the cysts measured up to 5 mm. in thickness, and were of a brownish-gray color; their internal lining varied from smooth to slightly rough. Small areas of red and yellow solid tissue were noted among the cysts. Attached to the mid-portion of the cystic mass, was a torn fragment of adrenal cortex which was grossly normal (Fig. 2B).

Microscopically, the walls of the cysts consisted of a fibrous capsule containing irregular islands and cords of adrenal cortical tissue. This tissue merged directly into gelatinous, slightly eosinophilic material, without intermediate structure in some of the cysts; in others, the cortical tissue appeared to be destroyed by extensive hemorrhage. Small cystic cavities were incorporated within the adrenal tissue and in many places the cortical cells were widely separated by blood-filled vascular spaces. The cells, both in the cystic mass and in the portion of normal adrenal cortex, showed a marked difference in size, shape, and nuclear pattern. Many of these cells appeared markedly enlarged with increased nuclear volume. The nuclear chromatin was arranged in large irregular clumps in which there were sharply contrasted spherical eosinophilic bodies. No mitotic figures or other changes indicative of a malignant process were found (Figs. 3A and 3B). The lesion was considered to be a hamartoma of the adrenal gland with secondary hemorrhage and cytomegaly within the walls of the cysts. The adrenal cortex was histologically normal.

*Described by Dr. H. Rappaport of Mount Sinai Hospital.

REVIEW OF THE LITERATURE

The first recorded case of adrenal cyst was reported by Greiselius[1] in 1670. The

Fig. 2*A*. Tumor mass intact with small left adrenal gland to the right.

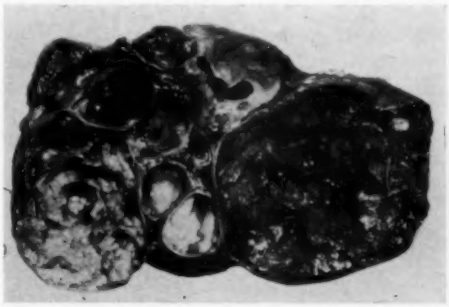

Fig. 2*B*. Cross section of tumor showing multiple cysts which contain hemorrhagic fluid, solid tissue, and various stages of degeneration.

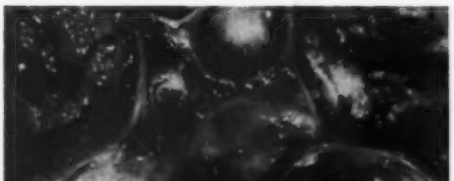

Fig. 3*A*. Magnification of cross section.

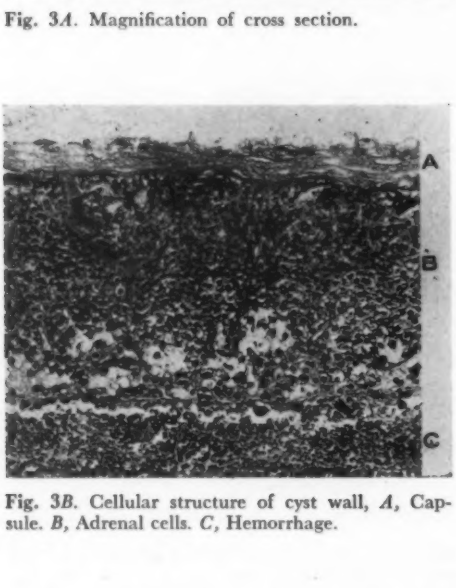

Fig. 3*B*. Cellular structure of cyst wall, *A*, Capsule. *B*, Adrenal cells. *C*, Hemorrhage.

tumor, found at autopsy, arose from the left adrenal gland. The cyst had ruptured, expelled more than 4 L. of blood-stained fluid and fetid clot into the free peritoneal cavity, and caused the death of the patient.

Reiman and Guyton[2] encountered only 4 cases in the American literature in the 20-year period prior to 1947; none was in children. Stock,[3] in the same year, found 50 recorded cases in the world literature. Wahle,[4] in 1951, recorded 9 cases in a series of 13,996 autopsies, an incidence of one in 1,555 cases. Abeshouse and co-workers,[18] in 1959, reviewed the literature and were able to find 152 cases, and added their 3 cases.

There is no unanimity of opinion regarding the etiology and pathogenesis of adrenal cysts. Terrier and Lecene,[5] and Ellis and others[24] classified cysts of the adrenal gland and proposed several subgroups as follows.

1. PARASITIC CYSTS

Parasitic cysts are mostly echinococcal cysts involving one adrenal gland, or are part of a generalized disease. These cysts have thick walls, daughter cysts, with or without calcification, and are associated with a positive Casoni skin test.[6-8]

2. EPITHELIAL CYSTS

Epithelial cysts are an uncommon variety of adrenal cysts and are divided into 3 subgroups:

A. True glandular. True glandular or retention cysts are extremely rare, because the cellular components of the adrenal gland do not exhibit the tendency toward acini formation.[26, 27]

B. Embryonal cysts. Embryonal cysts are lined with cylindrical epithelium and are caused by the displacement of tissue from the urogenital anlage with cystic transformation.[14]

C. Cystadenomas. Cystadenomas consist of an adenoma of the adrenal gland with secondary cystic transformation.[20-22]

3. ENDOTHELIAL CYSTS

Endothelial cysts represent the second most common variety found in adults. They can be divided into 3 subgroups:

A. Lymphangiectatic cysts. Lymphangiectatic cysts are very common, and result from ectasia of the lymphatic vessels normally present in the adrenal capsule and the medulla, or from incomplete linkage of these channels with the rest of the lymphatics. They are multilocular, contain clear or milky fluids, and possess a flat endothelial lining.[2, 13, 24]

B. Angiomatous cysts. Angiomatous cysts are rare and result from ectasia of blood vessels. On occasion they are associated with hemangioma of the liver or skull.[17, 20]

C. Hamartoma. Hamartoma is a rare

tumorlike malformation or fatal maldevelopment, in which various tissues of an organ are present in improper proportion.

4. PSEUDOCYSTS

Pseudocysts represent the most common clinically recognized adrenal cysts in adults. They are large cysts containing blood or reddish-brown-tinged fluid, with an irregular pigmented lining and connective tissue wall which may frequently contain calcium. They usually occur as a result of the following conditions:

A. Hemorrhage within normal adrenal tissue.[19, 24, 25, 27] The occurrence of adrenal hemorrhage in the neonatal period, or following meningococcemia, is well documented. Such a hemorrhage could undergo encapsulation and cyst formation.

B. Pseudocysts. Pseudocysts may also develop secondary to degenerative necrosis and hemorrhages within adrenal tumors, such as pheochromocytoma,[24] hemangioendothelioma,[10] or other adrenal tumors.[4]

Adrenal cysts in infants and children are rare. We have found only 11 such cases in the world literature. Table I summarizes the age, sex, symptoms, and type of cyst reported in the pediatric age group.

DISCUSSION

In our patient, the absence of adrenal insufficiency, the cytologic abnormalities, the presence of anemia, and the absence of an enlarged spleen or liver as centers of extramedullary erythropoiesis in the presence of severe anemia are all points of interest.

The 5-year follow-up without a history of acute or chronic adrenal insufficiency is evidence of the child's ability to maintain homeostasis with one adrenal gland.

The cyst in our case was a hamartoma, and is pathologically consistent with Albrecht's definition[15] which described it as a "tumor-like malformation in which various tissues of an organ are present in improper proportions or distribution with a prominent excess of one particular tissue." In the cyst described here both endothelial and vascular elements were involved.

The cytomegaly seen in the wall of the adrenal cyst (Fig. 4B), has not been described in any of the other instances of adrenal cyst which we have reviewed. Its significance remains in question in view of the

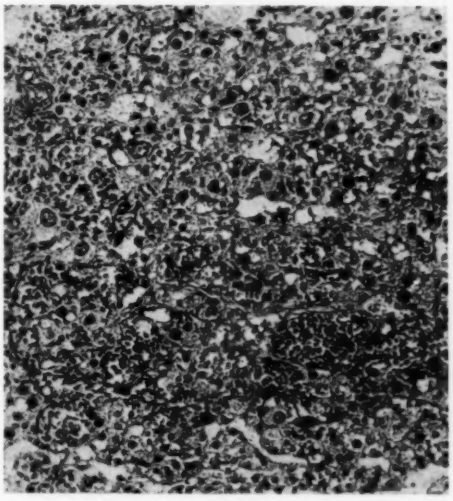

Fig. 4*A*. Cytomegaly, hemorrhage, and adrenal cells in the cyst walls.

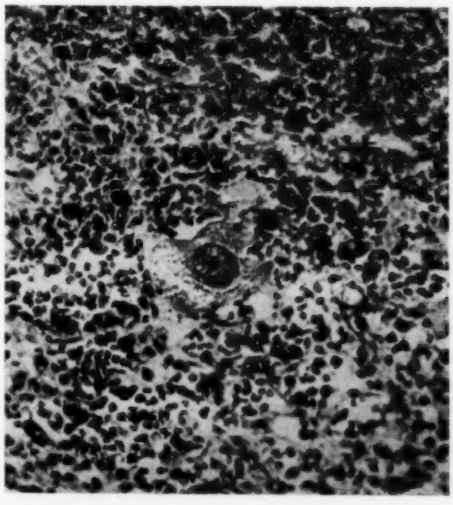

Fig. 4*B*. Cytomegaly in the hemorrhage wall.

finding of cytomegaly in the adrenals of both the fetus and the newborn infant.[16]

The anemia in our patient was not explained. In the absence of any prenatal or neonatal distress, slow intrauterine bleeding into the adrenal tumor seemed to be the most likely cause. The refractoriness of the anemia following operation despite the use of blood transfusions, however, requires further explanations.

The normochromic anemia, the lack of reticulocytosis, the absence of nucleated red blood cells in the peripheral blood, and the hypoplastic red cells in the bone marrow are similar to the findings in the nonhemolytic anemia of the newborn infant described by Smith.[32] These infants during their first weeks of life reveal hemoglobin and red cell values surprisingly lower than the average. The red cells are usually normochromic and normocytic, occasionally macrocytic, and no increase in the normoblasts over the normal values is found. The spleen and liver are not enlarged, and icterus, if present, is within the period of physiologic jaundice. These infants recover spontaneously or do well after small transfusions.

The differential diagnosis of an abdominal mass in a newborn or older infant should certainly include an adrenal cyst in addition to the more common abdominal tumors.[33]

SUMMARY

A case of an adrenal cyst which manifested as an abdominal mass in a newborn infant is presented. The clinical course and the pathology are discussed. A review of the related pediatric literature is presented.

We are indebted to Dr. Albert Richter of Mount Sinai Hospital for his assistance.

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TROPICAL PEDIATRICS

Neonatal pulmonary pathology in Singapore

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SINGAPORE, MALAYA

THE study of pulmonary lesions in the newborn infant seems, hitherto, to have been mainly confined to pulmonary hyaline membrane disease, and virtually all of the published work emanates from the Occident. Buckingham and Sommers[1] commented, in a review of Indian neonatal deaths, on the rarity of this disease in India and emphasized the possible importance of epidemiologic factors in its pathogenesis.

However, in Singapore, pulmonary hyaline membrane disease was the commonest cause of death in infants dying within the first week of life. This paper presents a histopathologic and statistical study of all the pulmonary lesions, including hyaline membrane disease, seen in a consecutive series of autopsies on such infants.

SINGAPORE: POPULATION AND VITAL STATISTICS

The State of Singapore is an island some 217 square miles in extent, situated 1 degree north of the Equator and 104 degrees east of Greenwich.[2] The climate is moist, hot, and equable. The average temperature is 85° F., and the average relative humidity is 83 per cent. There are no seasons.

The population is multiracial, 75 per cent Chinese, 14 per cent Malays, 9 per cent Indian and Pakistani, and 3 per cent others (Eurasian, European, Ceylonese, Arabian, etc.).[2] As in many Eastern countries, the Singapore population has a very low average age. By the 1957 census, 54.0 per cent of the one and a half million population were under 21 years of age.[2] The crude natural increase in 1958 was 35.0 per 1,000.[3] In the same year the crude birth rate per 1,000 mid-year population was 42.0[3] The infant mortality rate, defined as the number of deaths under 1 year of age per 1,000 live births, was 43.3.[3] This rate varies considerably with the various constituent ethnic groups, being 85.5 for Malays, 35.3 for Chinese, 40.3 for Indians and Pakistanis, and 44.2 for Eurasians,[2] reflecting the relative willingness of these groups to seek medical advice and hospital treatment.

The neonatal mortality rate, i.e., deaths under 28 days of age, was 19.0 per 1,000 live

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births. The perinatal mortality rate, i.e., deaths under 7 days of age plus stillbirths registered, was, in 1958, 29.0 per 1,000 live and stillbirths. In the same period deaths under 7 days were 14.1 per 1,000 live births. The stillbirth rate was 15.4 per 1,000 live births.[3]

Of the 63,572 live births in 1958, 31,510 or 49.6 per cent took place in government hospitals and 26,244 or 41.3 per cent in private homes.[3] The remaining 9.1 per cent took place in other hospitals and nursing homes. It will be noted that the perinatal and neonatal mortality rates are low, although the bulk of the babies born at the Kandang Kerbau Maternity Hospital (370 beds) are discharged about 24 hours after birth. It may be of interest to note that in this hospital, in 1959, the maternal mortality rate was 0.89 per 1,000.[4]

MATERIAL AND METHODS

In the Kandang Kerbau Maternity Hospital, Singapore, there were 38,114 live births and admissions during the period between June, 1959, and June, 1960. During this period there were 555 deaths in the hospital of newborn infants aged 1 week and below. Of these 423 cases were autopsied (Autopsy Index, 76.2 per cent).

The lungs of 356 patients were available for study. There was no selection. Ten per cent formal saline was used as the fixative. Paraffin-embedded sections were taken from both lungs and, in most cases, sections of an entire lung were mounted on a slide, stained with hematoxylin and eosin, and examined.

In each case one of the following lesions was recorded, by the use of the criteria mentioned below. For mixed lesions classification was based on the predominant histologic feature. Emphysema was omitted as it was believed in many instances to be caused by attempts at resuscitation with positive pressure oxygen.

Atelectasis. Lungs that were placed in this category showed varying degrees of collapse from complete cohesion of alveolar septi to expansion which was considered below normal. The cells lining the alveoli were low cuboidal in type. No distinction was made between primary and resorption atelectasis (Fig. 1).

Atelectasis with hyaline membrane. Lungs showing atelectasis, together with the presence of acidophilic membranes lining the alveolar ducts or alveoli, were included in this group. The hyaline membranes varied in thickness and length and were distributed diffusely throughout the lungs. Strands of fibrin associated with hemorrhage or pneumonia, and amorphous hyaline masses, the result of massive aspiration of amniotic fluid which were previously described as the "vernix membrane"[5] were excluded (Fig. 2).

Pulmonary hemorrhage. The criterion used was the presence of extensive intra-alveolar hemorrhage distributed throughout major portions of lung tissue. Scattered intra-alveolar or interstitial hemorrhages which were sometimes seen situated beneath the pleura were considered agonal, and these were not included (Fig. 3).

Pneumonia. The presence of more than a few polymorphonuclear leukocytes, whether

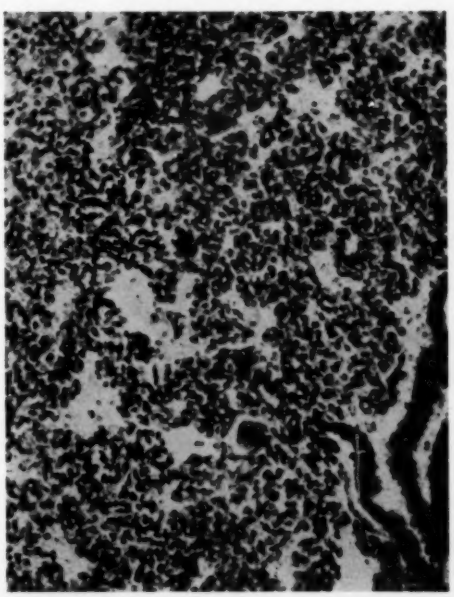

Fig. 1. Atelectasis.

Table I

Pulmonary lesions	*Weight*					
	0 to 500		*501 to 1,000*		*1,001 to 1,500*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Atelectasis and hyaline membrane			13	29.5	45	45.0
Atelectasis	1	100	9	20.5	10	10.0
Pneumonia			4	9.1	8	8.0
Pulmonary hemorrhage			1	2.3	12	12.0
Amniotic aspiration				–		–
Immaturity			7	15.9	1	1.0
Total No. of lungs examined	1		44		100	
Total No. of cases	3		44		113	

in the air spaces or in the interalveolar or peribronchial interstitial tissues was considered sufficient for a diagnosis of pneumonia. The exudates, in most cases, unlike pneumonia in later life, showed relatively little fibrin. In two cases discrete granulomatous lesions with polymorphonuclear infiltration were seen. These were probably the result of a pneumonia of intrauterine origin.

Immaturity. Lung immaturity is used descriptively and in conformity with the terminology of other observers[6] on the same subject. It is not synonymous here with viability or a birth weight of 1,000 grams or less.

Lungs appearing immature resembled those of very young fetuses. These showed histologically a layer of cuboidal epithelium lining underdeveloped air sacs which showed either incomplete expansion or collapse, together with a relatively large amount of loose interstitial connective tissue.

The immaturity of lungs seen in one patient weighing 1,000 to 1,500 grams and in another weighing 1,500 to 2,000 grams was unexpected.

Amniotic aspiration. Although traces of amniotic material could be seen in the lungs of infants in the first day of life, those showing an excess of squames and numerous hyaline staining masses filling the alveoli of major portions of the lungs were placed in this group.

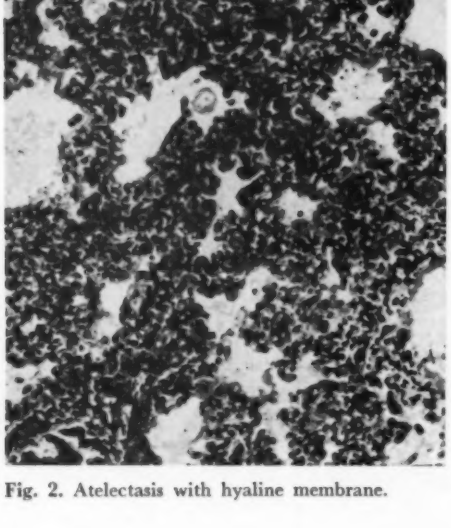

Fig. 2. Atelectasis with hyaline membrane.

RESULTS

Of the 356 newborn infants whose lungs were examined, 265 showed pulmonary lesions as follows:

Atelectasis with hyaline membranes	125	47.2%
Atelectasis	31	11.7%
Pneumonia	54	20.4%
Pulmonary hemorrhage	34	12.8%
Immaturity	10	3.8%
Amniotic aspiration	11	4.2%

Of the 423 cases that were autopsied, 115 had intracranial hemorrhage and 19 showed

(grams)

1,501 to 2,000		2,001 to 2,500		2,501 to 3,000		3,001 to 3,500		3,501 to 4,000	
No.	%	No.	%	No.	%	No.	%	No.	%
41	38.3	17	32.7	3	10.7	1	7.7	2	18.2
8	7.5	1	1.9	2	7.1	–		–	
14	13.1	13	25.0	12	42.9	2	15.4	1	9.1
12	11.2	4	7.7	1	3.6	2	15.4	–	
–		2	3.8	2	7.1	1	7.7	5	45.5
1	0.9	–		–		–		–	
107		52		28		13		11	
128		68		39		17		11	

gross congenital abnormalities. Intracranial hemorrhage was associated with a pulmonary lesion in 49 cases (20 cases, atelectasis with hyaline membranes; 17, pneumonia; 8, pulmonary hemorrhage; 2, amniotic aspiration; and 2, immaturity). The lungs of 32 patients with intracranial hemorrhage showed normal histology and those of 34 patients with intracranial hemorrhage were not examined.

Congenital abnormalities were associated in 7 cases with pulmonary lesions, and the lungs of 6 patients with congenital abnormalities showed normal histology, while in 6 cases the lungs were not examined.

Table I shows the distribution of pulmonary lesions in the different weight groups and Table II, the distribution of the same lesions during the first week of life.

DISCUSSION

Hyaline membrane disease in infants of the weight groups (500 to 2,500 grams) was found in 32.9 per cent of the autopsies performed in Singapore. This is the only series of cases of hyaline membrane disease reported in southeast Asia, China, and India. Only one case was reported in 1958 by Hadley, Gault, and Graham[7] in a review of stillbirths and neonatal deaths in south India. Latham, Nesbitt, and Anderson[8] reported the incidence of this disease in the United States to be 31 per cent of the autopsies performed on infants whose weights ranged from 1,000 to 2,500 grams.

Infants in the weight group of 1,000 to 1,500 grams showed the highest incidence. Other authors, e.g., Bruns and Shields,[9] and Avery and Oppenheimer[10] have also made the same observation. In the literature there has been some doubt expressed as to whether the smaller infants are liable to develop this disease, and it is believed to be uncommon in infants weighing 500 to 1,000 grams. Avery and Oppenheimer[10] for the same reason think that this underscores the association of prematurity and the disease. In our series the disease was not uncommon in the

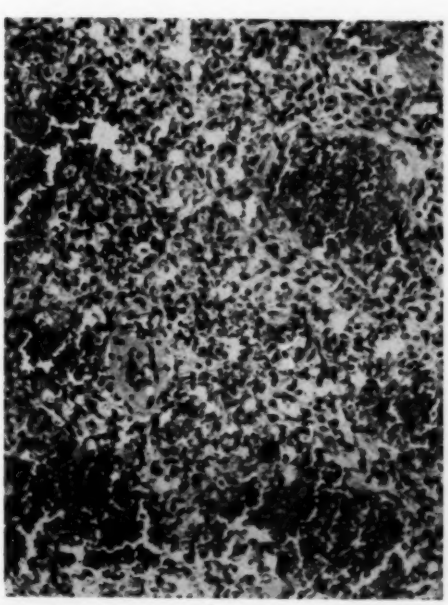

Fig. 3. Pulmonary hemorrhage.

Table II

Pulmonary lesions	*Age*					
	0 to 1		*1 to 2*		*2 to 3*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Atelectasis and hyaline membrane	75	41.9	37	35.6	11	42.3
Atelectasis	21	11.7	7	6.7	1	3.8
Pneumonia	18	10.1	18	17.3	3	11.5
Pulmonary hemorrhage	3	1.7	10	9.6	5	19.2
Amniotic aspiration	7	3.9	3	2.9	–	
Immaturity	7	3.9	3	2.9	–	
Total No. of lungs examined	179		105		26	
No. of cases	204		126		38	

weight group of 500 to 1,000 grams. (A statistical analysis of the 3 weight groups of 500 to 2,000 grams revealed no significant differences.) However, as the average normal birth weight here is less than in the United States, for a given weight, a Singapore infant will probably be functionally more mature and have a greater ability to survive than an American infant of equal weight. Thus, the weight group in question, 500 to 1,000 grams may be comparable with a group of greater weight in the West.

Atelectasis without hyaline membranes was responsible for the deaths of at least 31 infants. Although some might doubt atelectasis as a cause of death, this was the only lesion to which death could be attributed. Even in hyaline membrane disease, atelectasis appears to be the more important lesion and the hyaline membranes to be the "red herrings."[11] It has yet to be shown in the newborn infant with respiratory distress that hyaline membrane, rather than atelectasis, causes death by being a physical impediment both to the passage of air through the respiratory bronchioles and the exchange of

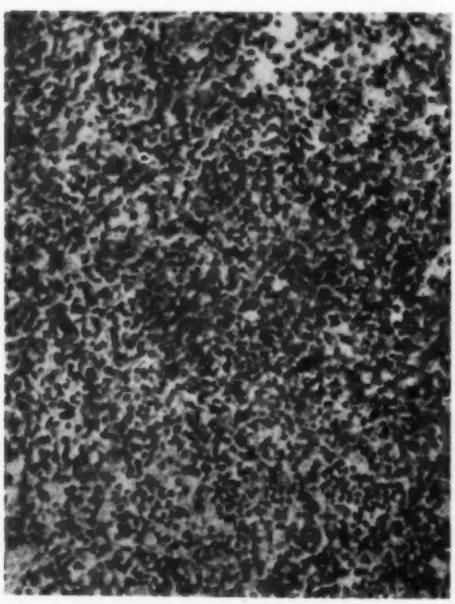

Fig. 4. Pneumonia.

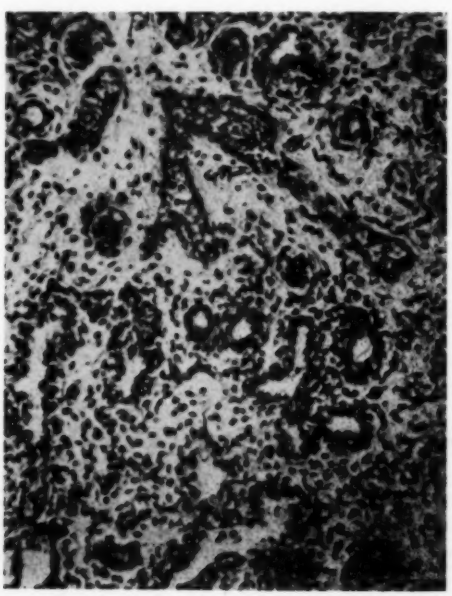

Fig. 5. Immaturity.

(days)

3 to 4		4 to 5		5 to 6		6 to 7	
No.	%	No.	%	No.	%	No.	%
2	10.5	0	0	0	0	0	0
2	10.5	–	–	–	–	–	–
6	31.6	2	20	1	11.1	3	33.3
4	21.1	1	10	5	55.6	6	66.7
–		–		–		–	
–		–		–		–	
19		10		9		9	
21		10		15		9	

respiratory gases in the air spaces. It is noted that both atelectasis and atelectasis with hyaline membrane were found to be more common in infants weighing 500 to 1,500 grams who died in the first day of life. There is probably some factor common to both conditions which has an etiological significance. Pulmonary hyaline membranes, without atelectasis, have been produced only experimentally, in animals.

The over-all incidence of pneumonia in the first week of life was 12.2 per cent of neonatal deaths (16.2 per cent of autopsies). Langley and Smith[12] found pneumonia in 27 per cent of neonatal deaths and Ahvenainen,[13] in 54 per cent. Most of the deaths due to pneumonia occurred between the third and fifth days of life, mainly affecting full-term infants. The low incidence of pneumonia in this series appears not to be in accordance with that noted by other observers. There is a low incidence in general of all infections in our hospitals. The early discharge of patients from the hospital, soon after delivery, does reduce the risk of cross infection. Other factors probably responsible for the low incidence of pneumonia are the equable climate and the relative absence of antibiotic-resistant strains of pathogenic organisms. The latter is probably the result of the restricted use of antibiotics here.

Extensive pulmonary hemorrhage which was considered sufficiently severe per se to cause death was found in 8.2 per cent of the

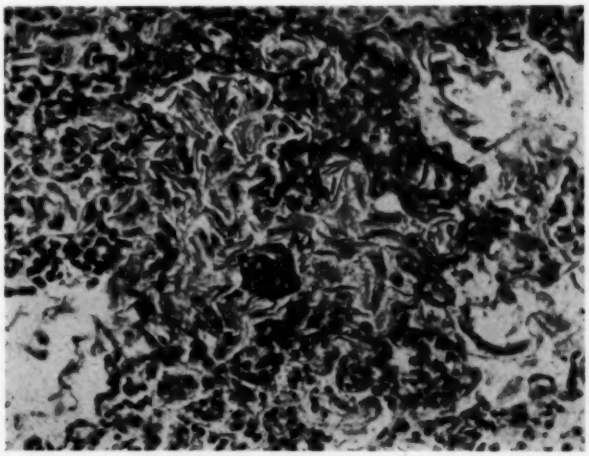

Fig. 6. Amniotic aspiration.

neonatal autopsies and was the third most important pulmonary cause of death. Many more cases showed moderate to slight hemorrhage which alone could not account for death. Some extravasation of red blood corpuscles was noted in the lungs of infants with atelectasis and hyaline membrane formation, but was not considered sufficiently severe to justify classification here. Laminated fibrin found associated with hemorrhage could be distinguished from true hyaline membranes. Only a few cases were seen in the first 2 days of life, and the increasing proportion of cases from the third day onward suggests that the greatly prolonged prothrombin time, rather than asphyxia, might have been responsible for the hemorrhage.

Marked aspiration of amniotic fluid was seen only in infants in the weight groups of 2,000 grams and above. The amniotic fluid of fetuses is free from or contains only low concentrations of epithelial squames and sebaceous material[14] Fetal distress evidenced by the aspiration of considerable amounts of amniotic fluid–contained material could, therefore, be expected only in the larger infants.

SUMMARY AND CONCLUSIONS

In the 423 neonatal autopsies at the Kandang Kerbau Maternity Hospital, Singapore, from June, 1959, to June, 1960, 265 showed pulmonary lesions. Of these, 47.2 per cent were characterized by hyaline membrane formation, this being the most frequent single cause of neonatal death. Hyaline membrane disease was found to be common in the smaller weight group (500 to 1,000 grams) of infants in whom it has been reported to be uncommon.

The incidence of this disease in tropical Singapore is comparable to the reported incidence in Western countries, suggesting that environmental factors are of little importance in the pathogenesis of hyaline membrane disease.

Pneumonia and pulmonary hemorrhage were also of importance, pneumonia affecting mainly full-term infants and having a relatively low incidence here. Pulmonary hemorrhage showed an increase from the third day of life onward.

Many thanks are due to Professor K. Shanmugaratnam for kind encouragement, the Director of Medical Services, Singapore, for permission to use data from the Kandang Kerbau Maternity Hospital, Dr. S. N. Kapur, Medical Superintendent who provided the same data, Dr. C. S. Muir for help in connection with statistical analysis, and Mr. V. Nalpon for the photomicrographs.

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MEDICAL PROGRESS

Some investigations on rickets

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GRONINGEN, NETHERLANDS

IT IS remarkable how little is known concerning the physiologic action of vitamin D. One of the main difficulties is the inability to determine the concentrations of vitamin D in different tissues. We know that vitamin D plays an important role in the metabolism of calcium, but since the store of calcium in the body is so large compared with its daily absorption and excretion, the delineation of its metabolism offers many difficulties. We know that vitamin D is necessary for adequate intestinal absorption of calcium. Administration of large doses of vitamin D results in an increased reabsorption of phosphate by the renal tubular cells and consequently in a rise of the serum level of phosphate. Another effect of large doses of vitamin D is an increase in the serum calcium level. Vitamin D deficiency, on the other hand, results in a decrease in the reabsorption of phosphate by the renal tubular cells. It is still uncertain whether the low level of serum phosphate in rickets is simply the result of decreased reabsorption of phosphate caused by the deficiency of vitamin D or if an increased activity of the parathyroid glands also plays a role. In addition to its effect on intestinal absorption of calcium and on renal reabsorption of phosphate, it is likely that vitamin D has a direct action on bone. In low concentrations vitamin D promotes deposition of calcium in bone.

The low levels of phosphate in the serum of rachitic children has attracted the interest of many investigators. Albright[1] has suggested that the high phosphate clearance in rickets is caused by hyperparathyroidism, which is a compensatory mechanism in response to the low serum levels of calcium, resulting from decreased absorption in the intestinal tract.

Others have suggested that vitamin D has a direct action on phosphate reabsorption in the kidney. Harrison[2] found that a vitamin D–deficient diet containing normal amounts of calcium and phosphate resulted in a low

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serum calcium level in the rat. The reduced serum calcium level could be raised only by parathormone and then only after vitamin D had been administered. It seems, therefore, that parathormone can exert its normal action only in the presence of vitamin D. There is additional evidence in favor of this concept, for example, rachitic children show a decreased response to administration of parathormone.

The renal absorption of still another group of substances is decreased in rickets. All patients with active rickets who subsequently responded to normal replacement doses of vitamin D demonstrated increased excretion of amino acids. This is especially true in the severe cases of rickets (Table I). In most instances the amino acid excretion was 2 or 3 times the normal value. The aminoaciduria is less marked on a diet low in protein when compared to a diet rich in protein and may easily escape detection (Table II). In all instances the amino acid excretion diminished after administration of vitamin D.[3] In some families rickets occurs more often than in other families. We found that the renal clearance of certain amino acids remained somewhat elevated in children of families who were prone to develop rickets even after the rickets had been cured.

We have observed this peculiarity in several families. It appears as if increased susceptibility to rickets and the need for more than normal amounts of vitamin D are associated with an increased aminoaciduria. We think that both peculiarities are hereditary and concomitant. In most instances some of the siblings and one of the parents of such a child show evidence of healed rickets. In these individuals the increased aminoaciduria disappeared only after 3,000 units of vitamin D had been administered for several weeks. If I am correct, we are dealing here with a hereditary susceptibility to rickets, a suggestion mentioned by authors of the period before vitamin D was known. There is a range of amino acid excretion; patients with moderate rickets are at one end of the spectrum and those with the de Toni-Fanconi syndrome or with the more severe forms of deprivation rickets (mainly with a hereditary component) are at the other end. Patients with resistant rickets occupy an intermediate position in this range. Not all patients with resistant or late rickets have increased excretion of amino acids. Recently we studied a family in which members of 2 generations showed signs of moderately severe vitamin D–resistant rickets with very high phosphate clearances and normal amino acid clearances.

It seemed worthwhile to find out which amino acids were excreted in increased amounts in our rachitic children[4]; the quantities of the different amino acids excreted were determined by the column chromatographic method of Stein and Moore. In all instances threonine, serine, glycine, alanine, histidine, and lysine were excreted in abnormal amounts (Table III).

Table I. Urinary excretion of alpha amino nitrogen of 9 patients with rickets

	Mean	
	Untreated	*23 days after one dose of 300,000 E. vit. D₂*
Serum P (mg. %)	3.0	5.9
Serum Ca (mg. %)	8.9	10.4
Phosphatase (K-A)	64.0	30.0
Urine		
Alpha amino N before hydrol. (mg./100 ml.)	32.0	15.0
Alpha amino N after hydrol. (mg./100 ml.)	81.0	34.0
Volume (ml.)	208.0	215.0

Table II. Influence of food uptake on the alpha amino nitrogen (amino acid) excretion*

	Normal		*Rickets*	
Alpha amino N	*Day*	*Night*	*Day*	*Night*
Urine (mg./c.c.)	0.11	0.09	0.15	0.12
N excretion (% of total)	0.99	0.90	3.3	1.8

*Alpha amino nitrogen excretion per milliliter of urine, body weight of the children 8 kilograms. The food with a protein content of 2 Gm. per kilogram body weight was given during the daytime.

Table III. The urinary excretion of some amino acids (mean of 3 patients). The values are given in milligrams per 24 hours

Amino acid	*Before vitamin D*	*After vitamin D*	*Decrease*	*Decrease (%)*
Threonine Serine	105.2	24.1	81.1	77
Glycine Alanine	95.5	57.8	37.7	39.5
Histidine	93.8	23.0	70.8	75.5
Lysine	53.5	32.2	21.3	40

Thus the excessive aminoaciduria is restricted to a limited number of amino acids which are normally found in smaller amounts in the urine of healthy children. Increased excretion of only a limited number of amino acids is also found in other disorders such as scurvy and cystinuria. But in these latter disorders different groups of amino acids are involved. In patients with the de Toni-Fanconi syndrome nearly all amino acids are excreted in abnormal amounts. The pattern of the amino acid excretion in severe resistant rickets is intermediate between that seen in vitamin D–deprivation rickets and that seen in the de Toni-Fanconi syndrome. Vitamin D given in large amounts decreases the excretion of amino acids in patients with severe resistant rickets or with the de Toni-Fanconi syndrome. Although the aminoaciduria of children with rickets may appear excessive, except in the case of histidine, it is small relative to the dietary intake. It seemed of interest to determine whether the aminoaciduria in rickets is caused by defective tubular reabsorption (that is, a lowered renal threshold) or by increased levels of amino acids in the blood so that the amounts delivered to the renal tubule by glomerular filtration are more than the normal tubules can reabsorb. Since the reabsorption of phosphate is defective in rickets, it seemed natural to attribute the aminoaciduria to failure of reabsorption of amino acids by the tubules.

Normal or slightly low level of free alpha amino nitrogen in the plasma is not proof of the renal origin of the aminoaciduria in rickets. The level of free alpha amino nitrogen in the plasma is the sum of the levels of the free alpha amino nitrogen of about 20 different amino acids. An elevated level of a few of these amino acids might hardly be detected if only the total plasma concentration of amino acids were obtained, especially since the levels of some amino acids might be low. It, therefore, seemed desirable to determine the levels of the different amino acids separately in the plasma ultrafiltrate of rachitic and normal children and to raise the level of certain amino acids in the plasma in order to study the amounts subsequently excreted. No major differences were found between the amino acid levels in the plasma ultrafiltrate of normal children and in children with different varieties of rickets. Before the rachitic children were treated with vitamin D, the response of their kidneys to histidine and arginine administered intravenously was tested.[5] Histidine was chosen because it is found in particularly large amounts in the urine of rachitic children; arginine, because it is excreted in strictly normal amounts.

In a normal child 3.5 per cent of the histidine and 0.3 per cent of the arginine given intravenously were excreted. In the rachitic

Table IV. Calcium and phosphate excretion after oral loading with phosphate in a patient weighing 8 kilograms. The phosphate load (1,200 mg. P) was given in the first 3 hours of the second 24 hours' period

	Before vitamin D			*After vitamin D*		
Ca per 24 hours (mg.)	2	2.5	3	6.5	4	13
P per 24 hours (mg.)	180	600	170	210	900	250
Urine per 24 hours ($cm.^3$)	120	163	220	216	300	350
	Excretion ± 30%			Excretion ± 50%		

child 32 per cent of the histidine given in this way was excreted, while the excretion of arginine was normal. Since the plasma levels of these amino acids during intravenous administration were about the same in both normal and rachitic children, the increased excretion must have been the result of decreased tubular reabsorption. Histidine clearance in the rachitic child was increased tenfold, whereas that of arginine remained unaltered. The decreased reabsorption of a limited group of amino acids in rachitic children must be the direct result of a lack of vitamin D in the renal tubules; administration of parathormone does not cause aminoaciduria. One is tempted to speculate as a result of these experiments that both the decreased reabsorption of amino acids and of phosphate in rickets are caused by the same metabolic defect. There are, however, patients with a hereditary form of resistant rickets who have increased phosphate clearances, but have normal clearances of amino acids. Increased excretion of phosphate and amino acids in rickets may not be caused by one metabolic block; the increased phosphate excretion might, unlike the amino acid excretion, be due to hyperparathyroidism.

There are other reasons to suggest that increased parathormone production may not be the main cause of the high phosphate clearance in rickets. Tetany with low serum calcium levels is sometimes seen in children in the early stages of rickets whose phosphate clearances are already increased. Some time ago we studied the problem of low calcium-tetany in rickets and we specially investigated the influences of phosphate loading on the serum levels of calcium and phosphate and on the excretion of these substances in the urine of normal and rachitic infants.[6, 7] We administered oral doses of 100 mg. P per kilogram in the form of phosphate to a number of rachitic children before and after treatment with vitamin D. Soon after the phosphate was administered the plasma phosphate level rose (Fig. 1). In the rachitic child the increase was somewhat less than that in the normal child. This was the result of a lower serum phosphate level in the rachitic child at the beginning of the experiment and of a somewhat higher phosphate clearance. In the normal child the calcium level remained fairly constant during the phosphate load, but in the rachitic subject there was a sharp decrease of the serum calcium level. The calcium level may drop so low that the child sometimes develops tetany. The tendency to decrease serum calcium levels in vitamin D–deficient children on a

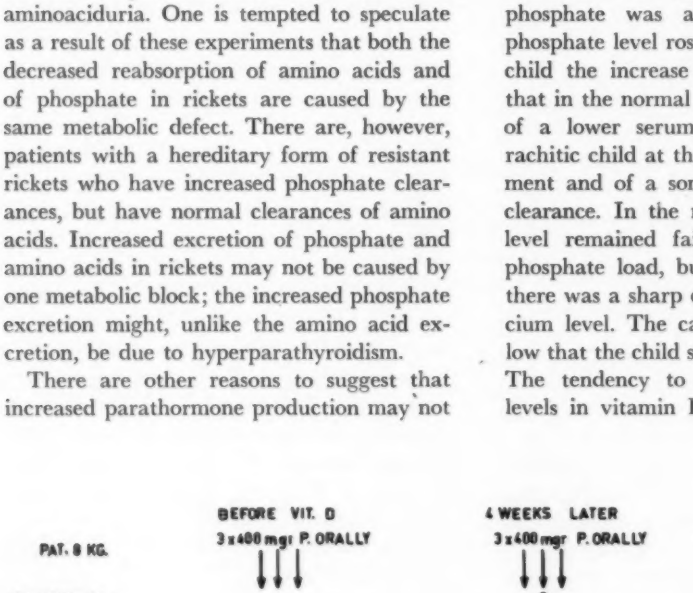

Fig. 1. Serum calcium and phosphate levels in a rachitic child during a phosphate load.

diet rich in phosphate or after a phosphate load may explain those forms of tetany that accompany rickets. This form of tetany was once common in the Netherlands, especially, in those children who were overfed with cow's milk, a diet rich in phosphate. The percentage of the phosphate load that is excreted in the urine by the rachitic child is somewhat lower than that excreted by the normal child in spite of the fact that the phosphate clearance of the rachitic child is somewhat higher than that of the normal child (Table IV). This may be caused by the low serum phosphate levels of rachitic children.

The decrease of the calcium level of plasma during phosphate loading leads to the supposition that calcium cannot be mobilized as rapidly from the bone of the rachitic child as it can from that of the normal child. It is unlikely that this is caused by lack of calcium in the rachitic bones because the vitamin D deficiency was of short duration. Roentgenograms of the bones of the rachitic children showed no signs of decalcification, except at the epiphysial lines.

It seems more likely that parathormone does not release calcium from the bone of rachitic children as promptly as it does in normal children. Parathormone given in a dose of 100 or 200 units during a phosphate load to rachitic children does not prevent the decrease in calcium level, while parathormone administered to normal children during a phosphate load causes a rise of the plasma calcium level. This leads to the conclusion that parathormone cannot exert its normal action on the bones of the rachitic child.

There are additional arguments for this hypothesis. Parathormone given in a dose of 100 to 300 units to the rachitic child results in only a negligible rise of the calcium level and a decrease of the phosphate level (Fig. 2). Contrary to what is happening in the normal child, parathormone in this dose results in only a very limited increased excretion of calcium and phosphate in the rachitic child (Table V).

We thought that the instability of the

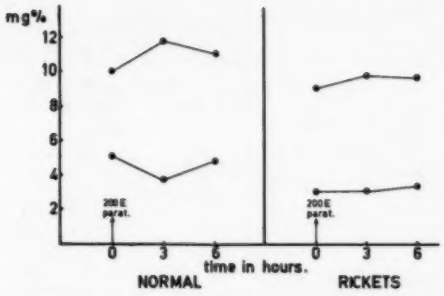

Fig. 2. The influence of parathormone on the plasma calcium and phosphate levels in normal and rachitic infants.

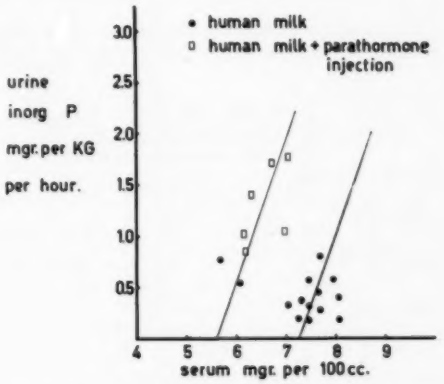

Fig. 3. Serum phosphate levels and phosphate excretion in breast-fed children and the effect of parathormone.

serum calcium in rachitic children might be used as a test for the diagnosis of developing rickets. Diagnosis of the early stages of rickets may be difficult, especially in the prematurely born infant. In such children roentgenograms of the bones are not very typical, since the first signs of rickets in these children are localized in the skull (craniotabes). The phosphate clearance should be higher than normal; we know, however, that in the young infant the phosphate clearance changes with the phosphate content of the formula. This makes it difficult to utilize increased phosphate clearances as an early sign of rickets. Increased excretion of a number of amino acids is of limited diagnostic value at this age, because many young in-

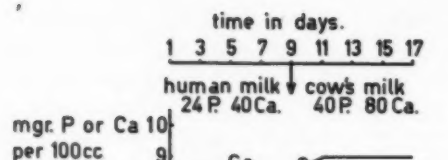

Fig. 4. The effect of a change from human milk to cow's milk on the serum calcium and phosphate levels.

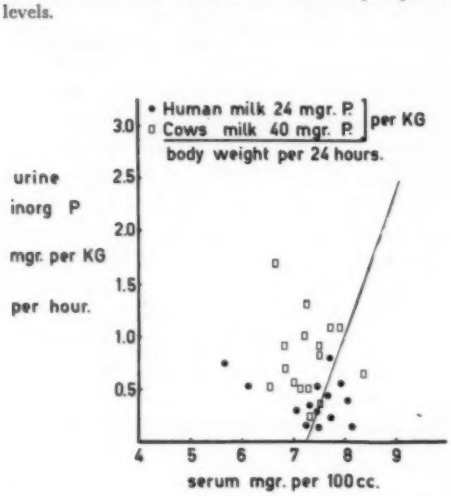

Fig. 5. Serum phosphate levels and phosphate excretion in children fed human and cow's milk.

fants and especially premature infants have increased excretion of amino acid (Table VI). Therefore, we tried to use a phosphate loading test to detect rickets in premature infants who received no vitamin D.

Immediately after birth the serum calcium levels and phosphate levels of newborn infants are somewhat higher than those of the mother. After birth the phosphate level tends to rise even in the breast-fed child although the phosphate intake is low.[8] At the same time the calcium level has a tendency to fall. In the breast-fed child the urinary excretion of calcium is low in most instances. There are, however, important individual differences. In those breast-fed infants whose urinary phosphate excretion is high, the blood phosphate level is low (Fig. 3). When a formula rich in calcium and phosphate is administered to a hitherto breast-fed infant, there is a temporary rise of the serum phosphate level (Fig. 4). The urinary excretion of phosphate increases and the blood phosphate level shows a tendency to fall, except in those instances where the glomerular filtration rate is low.

It is uncertain whether the increased phosphate clearance that occurs when a formula rich in phosphate is administered is a result of hyperparathyroidism. In most cases there is no rise of the blood calcium level or an increased excretion of calcium as would be expected in the case of hyperactivity of the parathyroid gland. The data in the literature on blood calcium and phosphate levels of children fed with cow's milk are rather contradictory.[9-11] Most authors agree that the serum phosphate levels of such children are generally higher than those of breast-fed children and that the serum calcium levels are nearly normal. Other investigators, however, found low serum calcium levels in children fed with cow's milk. In some instances even tetany occurred. These contradictory findings are not difficult to explain. When administration of phosphate has not been very high, increased excretion will compensate for an increase in the intake of it. When the phosphate load is increased, the infant will be able to deal with it only at the higher blood phosphate level. This is especially the case in the premature infant who has a low glomerular filtration rate. When the blood phosphate level is elevated (over 15 mg. per cent), the serum calcium level will decrease even in those infants who have received adequate quantities of vitamin D. Moreover, it is quite possible that some of the infants described in the literature had vitamin D deficiency and, therefore, had an unstable serum calcium level.

We have studied serum levels and the urinary excretion of calcium and phosphate in a number of prematurely born infants with

a birth weight between 1,500 and 2,000 grams. We started our studies when the children were about 1 week old. The infants fed with human milk received 40 mg. of calcium and 25 mg. of phosphate in the form of phosphate per kilogram of body weight, daily. The infants fed with cow's milk received a formula low in protein, calcium, and phosphate (Almiron A). The latter children received 100 mg. calcium and 50 mg. phosphate per kilogram of body weight, daily. The phosphate load was given as neutral sodium phosphate in 2 doses at an interval of 3 hours, the total dose being between 40 and 50 mg. of phosphate per kilogram of body weight. In the breast-fed children phosphate excretion was very low. The serum phosphate level was high in most instances (7 mg. per cent). The phosphate clearance, therefore, was low. There were, however, considerable individual differences (Fig. 3). In some cases the serum phosphate level was found to be low, whereas the phosphate excretion was found to be relatively high. In the children who received the cow's milk formula the serum phosphate level was only slightly more elevated than in the breast-fed children. The phosphate excretion was greater in the children fed with cow's milk, indicating higher phosphate clearances (Fig. 5). When the parathormone was administered to both groups, the serum phosphate levels fell and the phosphate excretion rose temporarily (Fig. 3). This prompt response to parathormone may have some therapeutic value in distressed newborn infants whose serum phosphate levels can rise to dangerously high values. Injection of parathormone will lower the serum phosphate level and will promote the buffer capacity of the urine at the same time. The response of a phosphate load is the same in infants fed either breast milk or cow's milk; there is a temporary rise of the serum phosphate level (Fig. 6). The serum calcium level does not show a tendency to decrease. Because of the moderate amount of phosphate that was administered in the loading experiment, the serum phosphate did not rise over 10 mg. per cent. In instances where a larger phosphate load was given, the

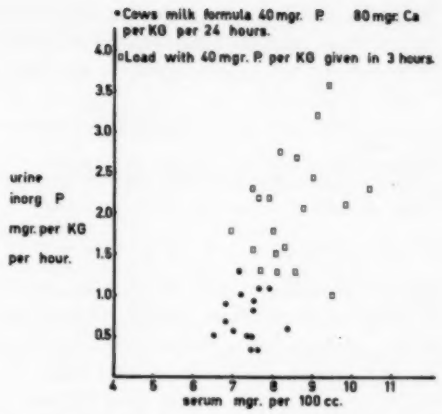

Fig. 6. Serum phosphate levels and phosphate excretion during a phosphate load.

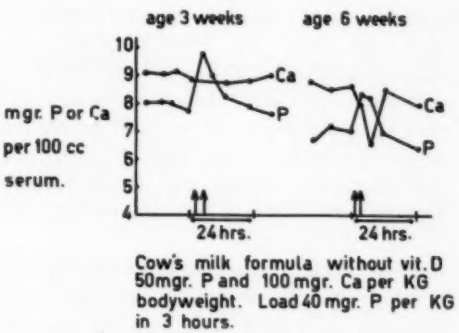

Fig. 7. The influence of a phosphate load on the serum calcium level in a premature infant (birth weight, 1,300 grams) before and after the administration of vitamin D.

serum calcium level did show a tendency to decrease when the phosphate level rose over 15 mg. per 100 c.c.

A number of premature infants who did not receive any vitamin D during the first 5 weeks of life were given a second phosphate load in the fifth week of life (3 weeks after the first phosphate load). Roentgenograms did not show evidence of rickets at that time. The serum phosphate level had decreased from an average of 8 to an average of 6.5 mg. per 100 c.c. Alkaline phosphatase activity was somewhat higher than at the time of the first loading test. The

Table V. The influence of parathormone (200 units) on the phosphate and calcium excretion of normal and rachitic infants (12 hours). The parathormone was administered at the beginning of the third 12 hours' period

	Normal				*Rickets*			
Ca (mg.)	15	12	20	45	2.5	3	1.9	4
P (mg.)	116	120	260	150	116	126	108	98

urinary amino acid excretion which is high in all premature infants had decreased somewhat less during that period than one might have expected. In many of the infants in this group the phosphate load caused a marked decrease in the serum calcium level, although the serum phosphate level rose to somewhat lower values than during the first loading experiment (Fig. 7). After the children had received 1,500 units of vitamin D daily for 2 weeks, the phosphate loading test was repeated. The calcium level of the serum had risen after the administration of vitamin D, and it remained constant during the phosphate load. In my opinion, therefore, the phosphate loading test described here may be used to detect early stages of rickets in infancy.

I would like to make a few remarks concerning the influence that a combination of factors such as food, sunlight, and skin color may have on a population in the long run. Children with darker skin need more sunlight in order to prevent rickets than children with a fair skin. This may be the reason that rickets is not rare among dark-skinned children in the tropics. We may assume that the dark pigment in the upper layers of the skin absorbs part of the ultraviolet light and reduces, therefore, the formation of vitamin D. If the dark-skinned child is more vulnerable to rickets than the fair-skinned one, and if ultraviolet light is the main source of vitamin D, and if the amount of vitamin D the child gets in this way is marginal for the prevention of rickets, then the fair-skinned individual will have an advantage in those parts of the world where the amount of ultraviolet light is very limited during a long period of the year. Under these conditions the mortality rate of the darker-skinned child due to rickets should be higher. Skin color is determined by one or more hereditary factors. In each successive generation of people living under conditions of limited sunlight a loss of genes for darker-skin color should occur. After many generations such a population will show lighter skin and fairer hair if it is supposed that in the original population a number of carriers of genes for fair skin and hair were present. These conditions have been present in northwestern Europe. The winters are long and for about 3,000 years or 150 generations the main food of the children during their first 2 years of life has been flour in the form of bread and pap made of flour and milk. Agriculture supplanted hunting and fishing as the main sources of food supply in northwestern Europe about 1,000 B.C. Fish may have been an important food for the older people, specially for the men, but the child when 1 year of age living mainly in an agricultural community will seldom receive enough fish to supply a reasonable amount of vitamin D. This may explain why the population of northwestern Europe and those whose ancestors originated from there have the fairest hair and skin of all people on earth.

Table VI. Alpha amino nitrogen excretion per kilogram body weight at different ages

Age	*Milligram per kilogram*	*% of total N excretion*
6 days	4.0	3.6
9 days	6.5	9.2
30 days	3.1	2.0
45 days	3.0	0.9
½ to 13 years	2.4	1.0

The fact that other ethnic groups who have lived, perhaps for even longer times, equally far north did not develop a fair skin can be explained by the fact that the food of their children was more varied and contained more vitamin D, because they were hunters and fishers instead of farmers and could not give bread and cow's milk to their children.

If one assumes that the gene frequency for fair skin in the population of northwestern Europe was 5 per cent and that the mortality rate of homozygous dark-skinned children was 3.3 per cent higher, and that of heterozygous ones was 1.6 per cent higher than that of those who were homozygous for the fair-skin genes, the frequency for fair-skin genes 150 generations later would be 95 per cent. If one takes into account the high mortality rate of all children in the past, such a small difference between those groups may not have attracted attention. The equilibrium between the amount of ultraviolet light that reached the skin, the amount of vitamin D in the food, the skin color, and the occurrence of rickets must have been very labile in northwestern Europe. Small changes in the way of life or in climate can explain the outbreaks of rickets which occurred in the first half of the seventeenth century. Vitamin D is now available for each child who needs it and this means that vitamin D deficiency need no longer be a factor in the history of mankind.

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CLINICAL PATHOLOGICAL CONFERENCE

THE CHILDREN'S HOSPITAL MEDICAL CENTER, BOSTON, MASS.

Sidney Farber, M.D., Editor

Gordon F. Vawter, M.D., Assistant Editor

THIS was the first Children's Hospital Medical Center admission of a 4½-year-old male, with the chief complaints of anemia and chronic pulmonary disease.

The patient was essentially well until April, 1957, when, at the age of 2 years, he was admitted to another hospital because he had passed several worms and because of low hemoglobin and low hematocrit values discovered at the time of a routine check-up. In the hospital, laboratory studies showed: hemoglobin 34 per cent; hematocrit, 19 per cent; reticulocytes, 2.4 per cent, white blood cell count 11,700 per cubic millimeter; and platelets normal. The bilirubin was 1.3 mg. per cent, all of which was indirect reacting. The serum protein comprised an albumin concentration of 4.5 Gm. per cent and a globulin concentration of 2.1 Gm. per cent. Transfusion was done twice and the child was discharged on the eighth hospital day. Following discharge, he had transient epigastric and upper abdominal pain and some frontal-parietal headaches. The hematocrit fell over the next 2 weeks to 29 per cent, and it was noted that he had a reticulocyte count of 6.7 per cent.

One month after discharge he was readmitted to the hospital, again with a low hematocrit and elevated reticulocyte count, but also with some respiratory distress. X-ray examination showed diffuse bilateral interstitial infiltration. On the ninth day of that admission the hematocrit had dropped 5 per cent and he was given another transfusion.

Laboratory data at this time revealed a normal urine urobilinogen. Stools were negative for occult blood. Urine coproporphyrins were negative.

Direct and indirect Coombs tests were negative. Clotting studies were normal. Electrophoresis showed no evidence of an abnormal hemoglobin. Barium enema and upper gastrointestinal series were normal, as were x-ray films of the skull, legs, and intravenous pyelogram. Cold agglutination tests were negative. Tuberculin tests in the first and second strength PPD (purified protein derivative) were negative, as were skin tests with histoplasmin, coccidioidin, and Blastomycin. Electrophoresis was normal, and a lupus erythematosis preparation was negative. The child was subsequently discharged

on Meticorten and ferrous sulfate. He was kept on Meticorten for approximately 2 years, after tapering of the Meticorten to 2.5 mg. twice a day.

When he was admitted in April, 1960, his parents said he had gained weight poorly and had episodes of vomiting, with a few blood streaks but no massive hematemesis. There had been some loose bowel movements, and repeated chest x-ray examinations had demonstrated some infiltration bilaterally. The past history was otherwise noncontributory, as was the family history. Physical examination showed a temperature of 98° F., pulse 100 per minute, respirations 18 per minute, and blood pressure 90/50 mm. Hg. The weight was 30 pounds, height 37.5 inches (both below the third percentile). He was small and undernourished, but not in any acute distress. The skin turgor was good and the sclerae were clear. The lungs were clear to percussion and auscultation, and the heart showed no enlargement. There was a normal sinus rhythm. The liver edge was 2 cm. below the right costal margin; the spleen was not palpable. Neurological findings were entirely within normal limits. Nose, throat, and ears were clear.

Laboratory data revealed a normal urinalysis, hemoglobin 12.4 Gm. per cent, and white blood cell count 16,800 per cubic millimeter, with a normal differential. Reticulocytes were 4.5 per cent, platelets 368,000 per cubic millimeter. Nonprotein nitrogen was 23 mg. per cent, albumin 4.3 Gm. per cent, globulin 2.3 Gm. per cent, serum glutamic oxalacetate transaminase 17.5 units, and bilirubin 0.3/1.3 mg. per cent. Thymol and cephalin flocculation tests were negative, and cholinesterase 51 units. Old tuberculin skin test (1:2,000) was negative. Stool guaiac was negative. Electrophoretic pattern was entirely within normal limits. Examination of the chest by x-ray showed the lungs to be slightly increased in volume and throughout each lung there was a flocculent, confluent, hazy density, with no visible fine granular infiltration. The costophrenic angles were clear. The heart and great vessels appeared within normal limits. After observation and review of the chest x-rays, an operation was performed.

Dr. Kenneth T. Bird.* In short, this is a 4½-year-old child who was found, at the age of 2 years, to have a severe anemia and a rather unexpected diffuse bilateral pulmonary infiltration. I say "unexpected" since (1) there were no pulmonary symptoms, no cough, no sputum, and no wheezing, and (2) because no abnormal physical findings were produced in the lung. I will promptly exclude consideration of both serum hepatitis in a child who has received transfusion and transfusion siderosis where, although the main site of storage is the liver, hemosiderin may accumulate in other organs. The anemia was apparently insidious in onset because the first hospitalization was precipitated by the fact that he had passed several worms, rather than by the severe anemia which was then discovered. The exact nature of this anemia cannot be delineated but it would appear to be of the hypochromic variety, with the mean corpuscular hemoglobin concentration distinctly reduced. This anemia was accompanied by a reticulocyte count which remained elevated throughout the course of the illness and ranged from 4.5 per cent to even higher. However, the stool guaiac tests were negative, so that blood loss via the alimentary tract was virtually excluded. Since the Coombs test, both direct and indirect, was negative, certain of the acquired hemolytic anemias have probably been excluded or could temporarily be shelved. To settle the point further, we are assured by the protocol that the clotting studies were normal, electrophoresis showed no abnormal hemoglobin, and cold agglutinins were negative. We are told, by implication, that there was no family history of anemia. Moreover, the white cell count and platelet counts were essentially normal. In short, there was a blood loss or blood destruction type of anemia, without evidence for either. Could

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this be an acquired disease of chemical or toxic origin? I imagine we always have to consider lead poisoning in such a child with anemia; the classic symptoms should involve the gastrointestinal tract, the nervous system, and the blood. At the time of the first admission he had transient epigastric and upper abdominal pain. Lead poisoning can lead to anorexia and vague abdominal discomfort, or even severe colicky pain, which may be associated with segmental spasm of the musculature of the large bowel. Children are especially prone to central nervous system involvement, and yet this patient had only some mild degree of frontal-parietal headache. Finally, there is usually a moderate anemia with a certain degree of reticulocytosis in the presence of stippling. Absence of stippling does not exclude lead poisoning but a high reticulocyte count is strong evidence for active lead poisoning. I shall exclude lead poisoning because the coproporphyrin excretion is normal, and because lead poisoning does not cause pulmonary lesions, even though the lungs can serve as the portal of entry of lead into the body.

What about an acquired disease of parasitic origin, since at 2 years of age he passed several worms? Both flat and round worms may traverse the lungs in their complicated life cycles, producing transient pulmonary symptoms and infiltrations. Loeffler's syndrome, with episodic asthma, has been associated with various parasitic infections. On the other hand, certain flat worms, such as *Paragonimius westermanii, Echinococcus granulosa,* and schistosoma, may establish permanent residence within the lung parenchyma, or the supporting structures, and give rise to persistent symptoms and to persistent x-ray findings. Unfortunately, these x-ray findings are not specific. We can, I think, quickly exclude such flat worms as a cause of permanent pulmonary lesions in this case since the likely worms in a child of this age are round worms which tend to migrate *through* the lungs. Pulmonary infestation by round worms can be suspected when eosinophilia occurs with a usually transient bronchopneumonia in a person who is passing worms or ova in the stool. Sputum examination may show ova or larval stages, or even adult worms, depending upon the variety of nematode that produces the pulmonary symptoms. The anemia of hookworm infestation is often this marked, but I will liken the history of worm-passing to the serpent in the garden of diagnostic temptation. To avoid any further temptation, I will exclude the possibility that the worms and the rest of the symptom complex in this patient were parts of a single process. I think the worms may have uncovered the underlying process. From my point of view, I would be particularly interested in knowing the exact nature of the pulmonary lesion in the x-ray and whether or not the bilateral pulmonary infiltrations fluctuated at all, in appearance.

Dr. Harris. I am told that the previous films, made elsewhere, which we do not have, showed a gradual progression in the degree of involvement of the lungs. In the single chest film made here, multiple areas

Fig. 1. Hazy confluent densities are seen throughout both pulmonary fields. There is no hilar lymphadenopathy or abnormality of the cardiac silhouette.

of rather fluffy infiltration or exudate involve, to a considerable and symmetrical degree, portions of both lungs (Fig. 1). There is nothing particularly specific about the distribution or the general appearance of these shadows, and a host of etiological agents can produce the same appearance. The heart is within normal limits. There is no pleural fluid and the visualized bones in the chest look normal. There is no hilar adenopathy.

DR. BIRD. There seems no particular sparing of any part of the lungs on the posteroanterior view; this seems to be a universal process. Well, one picture is worth a thousand words. I had a little different visualization of what the x-ray might show.

For the purpose of discussion, we should note that sarcoidosis should always be considered in a patient with extensive bilateral pulmonary infiltration and negative tuberculin tests. The characteristic hilar, right paratracheal lymph node enlargement may actually not be present in later stages of the disease, but except for the abnormal and nondiagnostic chest films, there is no evidence to suggest chronic sarcoidosis in this patient. The serum globulin and gamma globulin levels were normal, but these are elevated in 75 to 80 per cent of patients with sarcoidosis. The visualized bones were normal, with no punched-out areas. There was no other peripheral adenopathy or skin lesions which may be present in up to 20 per cent of patients with sarcoidosis. In short, there is no evidence for such a granulomatous disease elsewhere in the body. Therefore, we cannot seriously consider that this is sarcoidosis with the rather unusual but well-documented association with an anemia which is usually hemolytic when it occurs and which is ordinarily secondary to sarcoid involvement of the spleen, for which we have no evidence here. Could this be a collagen disease? In passing, I would think of periarteritis nodosa, disseminated lupus, or even scleroderma. Of the 3, scleroderma should be mentioned other than by name. Chronic pulmonary changes, I think, are more possible in scleroderma; the pulmonary lesions are more often silent, yet this patient obviously had no sclerosis of the derma, either focal or diffuse. The extensive pulmonary involvement by scleroderma usually leads to progressive dyspnea, not a feature in this patient. So despite the extensive lung involvement in this youngster, since he had virtually no pulmonary symptoms and since the age group is not particularly compatible, I would like to exclude the possibility of one of the collagen diseases producing this type of total clinical picture. I think I should mention eosinophilic granuloma, specifically. There are many synonyms, including histiocytosis X, pulmonary xanthomatosis, Letterer-Siwe disease, and Hand-Schüller-Christian disease. Dr. Farber, as you all know, was the first to postulate that all these conditions represent variations in the degree and stage of development and localizations of the same basic disease process. Eosinophilic granuloma is characterized, microscopically, by endothelial cell proliferation and localized collections of eosinophils in the tissues, not in the blood, with associated abnormal storage of lipoid material. The skin, mucosa, lymph nodes, liver, and spleen may be involved. There may be multiple skeletal defects. The pulmonary lesions which I have seen in adults with eosinophilic granuloma were multiple, bilateral, and fairly symmetrical. Eventually they cause a definite full-blown alveolar capillary block type of syndrome. None of these are present in this patient and I think these possibilities unlikely. The question is, then, is there a specific disease which may begin in childhood, become chronic, possibly a recurrent process with pulmonary changes overshadowed by an anemia which becomes quite marked? Well, "brown induration of the lung" was described over 100 years ago by Virchow and clearly delineated in 1931 by Ceelen.[1] It was pointed out that alveoli were crowded with large cells covered with pigment, which possibly was hemosiderin. By 1936, 33 cases had been reported in the English literature, of which 30 were in children. By 1955, over 80 cases had been reported in all languages.

The disease usually starts in childhood.

When it is finally recognized, it becomes apparent that such children have often been "sickly" from birth. Anemia is often the presenting symptom. The over-all incidence, regardless of age, is probably equal in both sexes but in childhood, the disease appears somewhat more often in girls. Classically, the course is characterized by recurrent episodes of dyspnea, cyanosis, and cough, often with hemoptysis, fever, tachycardia, and anemia. The patient being discussed today does not fall into this classic pattern but there is a wide range in the clinical spectrum of this disease. Jaundice may occur. Most of these individuals have a degree of reticulocytosis. Furthermore, all studies of bleeding, clotting mechanisms, and iron metabolism have been within normal limits. Capillary fragility is also within normal limits. The anemia is of the iron deficiency type and when the serum iron has been determined, it is frequently low. Urobilinogen has been elevated in some of these cases. The anemia may even disappear in some of the chronic cases and be supplemented by a polycythemia, particularly if irreversible underlying pulmonary fibrosis results from the basic disease process. The appearance of the chest x-ray varies with the stage of the disease. Diffuse and confluent densities may be noted. In its more classical appearance, the disease spreads out from each hilum into the mid- and lower-lung fields and has quite a homogeneous appearance, not like these films, but such changes as are shown in the film have been described and seen in patients with this disease process. At times the pulmonary changes may be suggestive of pneumoconiosis, and this is particularly important since the disease has recently been described in adults who were previously in perfectly good health. Hilar adenopathy is uncommon. Pathologic findings in this disease are limited to the lungs, in which the alveoli are literally "stuffed" with blood. Fresh and old hemorrhages are seen, with many hemosiderin-filled macrophages. There is no gross structural lung abnormality or evidence of primary heart disease. The blood within the alveoli undoubtedly produces the hemoptyses, which are frequently an important aspect of this disease, but the disease can occur without hemoptysis.

Iron therapy may be beneficial, and steroids may alter the course favorably. This is not proved, however. The diagnosis can be documented by the finding of hemosiderin-containing macrophages in the sputum, provided mitral stenosis, bleeding gums, or blood dyscrasias are eliminated. Fasting morning gastric aspirates may also show hemosiderin-laden macrophages in this disease. This is a good point to remember in the child who does not expectorate. For a specific diagnosis, direct needle aspiration for needle biopsy of the lung has been used. I would not favor this approach. In this patient I would suggest that the operation to confirm the diagnosis was probably an open thoracotomy with lung biopsy.

My impression is that this patient will have changes consistent with idiopathic pulmonary hemosiderosis.

DR. GORDON F. VAWTER. Anti-A and anti-B titers were done, I think, to evaluate the history of passage of worms. These titers were strictly within normal limits.

DR. CHARLES D. COOK. This child, unfortunately, was too small for pulmonary function studies. Such data might have helped to pinpoint any abnormalities of respiratory function which might have been present. I think blood oxygen tension should have been determined since, as I remember, there was suggestion of some cyanosis, not definitely obvious. Most interesting of all would have been studies of pulmonary diffusion of oxygen, carbon dioxide, and carbon monoxide, which as a test gas may be utilized to study diffusion since these functions may be impaired in pulmonary hemosiderosis.

I think many of us remember a case that was presented here a year or so ago with an x-ray picture somewhat like this, and with a negative tuberculin. On lung biopsy there was definite evidence of miliary tuberculosis. I don't think such a process can be excluded, although I think it is a very unlikely possibility. Idiopathic pulmonary fibrosis in the early phase or, I should say, the Hamman-

Rich syndrome with alveolar capillary block, should be considered.

DR. LOUIS K. DIAMOND. This child did have gastric washings for tuberculosis with negative results. More extensive hematologic studies than are recorded indicated that this child had a hypochromic and microcytic anemia, rather than the hyperchromic state implied by the only figures supplied.

DR. BIRD. On the last admission he did not have very severe anemia.

DR. CHARLES A. JANEWAY. He had been receiving corticoids for 2 years.

DR. VAWTER. The absence of massive hemoptysis is probably reason enough for not doing a bronchoscopic examination to rule out angioma or other intrinsic lesions.

DR. DIAMOND. Dr. Bird, I wonder if you have any idea why, in children with hemosiderin in the lungs, the iron deposits seem to be more stable, in contrast to broken-down hemic pigments occurring elsewhere in the body? This would seem related to the hypochromic anemia which is unlike the usual response to a hemolytic picture, unless there is also external blood loss or complicating iron deficiency or defective utilization. Are these erythrocytes somehow excluded from the circulation in a special way, or is there a defect in the whole iron binding process, involving specific globulins or something else?

DR. BIRD. You have nicely pinpointed a most fascinating aspect of pulmonary hemosiderosis. Let me say that virtually nothing is known about the exact pathogenesis of this disease. All methods of analysis indicate considerable increase in iron content in these lungs, and there are several theories which attempt to account for this feature. One revolves around a possibility of vascular shunts at the level of pulmonary precapillary arterioles with development of bleeding telangiectases between pulmonary and bronchial circulation. This remains unconfirmed, partly since many such patients show none of the pulmonary hypertension which one might expect to be present. Another theory leans toward the possibility of an autoimmune mechanism. Another hypothesis relates

Fig. 2. Low-power view of serial blocks through a biopsy of the lung. The dark masses represent hemosiderin deposits. (Hematoxylin and eosin. Original magnification ×15.)

to the existence of some deficiency in the structure of the pulmonary capillary. Most of these ideas imply that the build-up of pulmonary iron stores is largely a matter of repeated petechial hemorrhages of sufficient amount to exceed the capacity of the mechanisms for removal. More recently, an hypothesis has been advanced concerning a specific abnormality in the function of the cells lining the alveolar septa.*

DR. DIAMOND. We have followed 2 patients with idiopathic pulmonary hemosiderosis treated with corticosteroids for prolonged periods of time. There is no good evidence in these 2 that the hemolytic anemia has responded in the dramatic way that may typify the response to steroids seen in other acquired hemolytic anemias. Also, we have no clear idea whether these 2 patients have benefited in any other way.

DR. COOK. This may be true from the hematologic standpoint, but some of these patients develop considerable pulmonary fibrosis, which one wants to prevent, if possible.

DR. BIRD. It is true that in acute pulmonary hemosiderosis, there is often only accumulation of iron, whereas if the bleeding is persistent or if the basic disease is, for one reason or another, persistent, irreversible pulmonary fibrosis may occur. In some re-

*Program of the Fifty-eighth Annual Meeting, American Association of Pathologists and Bacteriologists, 1961, p. 24.

pórted cases, in adults, the pulmonary picture may occasionally regress to a normal x-ray appearance, although this does not entirely exclude a degree of interstitial pulmonary fibrosis—and, indeed, some persisting degree of functional alveolar-capillary block has been described under these circumstances.

Certainly there is nothing worse than irreversible pulmonary fibrosis and we may, when faced with this, perhaps derive some cerebral benefits, if no other, from corticosteroid treatment. This is a point to consider in view of the usual fatal termination of the illness. It may well be that some of the instances of that poorly defined group of interstitial pulmonary fibroses are examples of burned-out idiopathic pulmonary hemosiderosis.

DR. VAWTER. One or two of the original cases of pulmonary fibrosis described by Hamman and Rich[2] had considerable alveolar hemosiderosis. Moreover, experimental pulmonary venous stenoses have produced a syndrome somewhat resembling both pulmonary fibrosis and pulmonary hemosiderosis. Mediastinal fibrosis in humans has occasionally led to similar complications.

DR. ELF E. GUNDERSEN. Exploration of the pleura and lung through a small right thoracotomy at the time of pulmonary biopsy revealed no gross abnormality in this patient.

DR. VAWTER. Our gross impression of the biopsy was that of a normal lung until it was cut in several areas, thus revealing many small brownish nodules in the soft lung.

Microscopically, nodules of hemosiderin-packed macrophages filled groups of alveoli, both peribronchially (where there was also a mild chronic bronchitis) and subpleurally, too (Fig. 2). One good-sized bronchial artery contained a small fibrous intimal plaque. Hemosiderin also outlines some of the fibroelastic tissue structures of alveolar septae, as has been quite typical in cases of *idiopathic pulmonary hemosiderosis*.[3] A few unidentified crystals with associated foreign body reaction were noted in one area. Giant cell response to calcified fibers, possibly elastic tissue, also is present and has been described

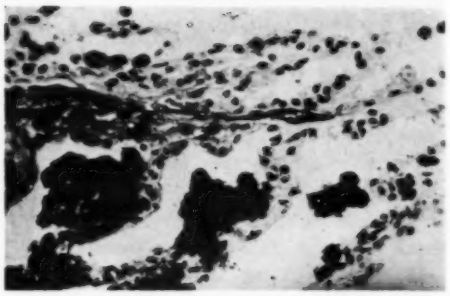

Fig. 3. High-power view of biopsy of the lung. The black masses represent hemosiderin-laden cells. Crossing the upper middle of the field are linear black streaks representing calcified elastic lamellae in a tiny pulmonary vessel. (Hematoxylin and eosin. Original magnification ×500.)

in pulmonary hemosiderosis (Fig. 3). Abnormalities of pulmonary vessels (such as angiectasis or gross defects in vascular walls) and gross deficiencies in elastic tissue, such as has been described in late idiopathic pulmonary hemosiderosis at the time of autopsy, were not recognized. Although no fresh hemorrhage is seen, the picture is compatible with petechial hemorrhage at scattered points. No evidence of pulmonary fibrosis is evident in these sections.

Dr. Sui Moto, from the Beth Israel Hospital, has brought some slides from a young boy, known to many of you, who died with brown induration of the lungs a few years ago. Actually, this boy was one of the first in whom the diagnosis was made after needle biopsy of the lung.

DR. SUI MOTO. Unlike the patient discussed today, our patient shows very firm, almost hard, indurated, grossly brown lungs at the time of death. The lungs are very heavy. Microscopically, there is massive interstitial pulmonary fibrosis, as well as marked pulmonary hemosiderosis. Hilar lymph nodes are also considerably enlarged and filled with hemosiderin pigments. There were no other essential changes found at autopsy, apart from evidence of pulmonary osteoarthropathy and signs of cor pulmonale associated with terminal progress in respiratory insufficiency and acute bronchitis.

DR. VAWTER. Dr. Bird, what in your opinion, might be the meaning of the differences in the pathologic pictures in these 2 cases? Are these different diseases; are these merely different stages in the disease; can one suspect a differing prognosis?

DR. BIRD. First of all, can we agree that the boy under discussion has idiopathic pulmonary hemosiderosis?

DR. VAWTER. There is pulmonary hemosiderosis for which we have no known cause. At one time or another it was associated with episodes of significant anemia. Whereas the lung is not now fully laden with iron, this deposit probably must be acquired by degrees and it seems likely that we are dealing with such a disease in an early stage, or of a milder than usual or classic type.

DR. BIRD. We could feel much more secure in choosing between the various possibilities if we had the chance to see serial x-rays. Bronchopneumonia associated with aspiration of blood is indistinguishable clinically and sometimes radiologically from other bronchopneumonias, since it may be accompanied by a chill, fever, leukocytosis, and shift of leukocytes to the left, with a soft, fluffy, radiologic shadow. Also, these cases are more common than idiopathic pulmonary hemosiderosis. Pneumonia secondary to aspiration of blood usually clears up quickly, with resolution in 7 to 10 days or so. In short, we are indeed dealing with idiopathic pulmonary hemosiderosis which I can only agree is mild in its current pathologic manifestations. The lack of more widespread elastic tissue alteration might indicate that such changes, described in the literature, may be only secondary abnormalities. This further weakens one theory of the basic nature of the vascular abnormality which leads to bleeding, namely, a primary elastic tissue defect.

DR. DIAMOND. We saw today a boy who, we believe, has had idiopathic pulmonary hemosiderosis, and who has been followed for 12½ years with serial x-rays. The pulmonary infiltrations have not seemed to change much, except that the lungs have increased in size around them.

As a small child he had one or two attacks a year, but in recent years he has been quite free of symptoms. One wonders whether the difference between our patients and the boy discussed by Dr. Sui Moto lies in the amount or type of secondary bacterial or other infections he had. The boy we have been following for 12 years has been put to bed with every little cold or respiratory infection, put on antibiotics, and is not allowed to resume activity until we think bacterial cultures are innocuous. We have kept him on iron therapy and recently the hemoglobin has been ranging between 14 and 16 Gm. per cent.

DR. JANEWAY. How often do you see this disease begin in adult life?

DR. BIRD. Often enough to promptly consider the diagnosis in the young adult who presents with hemoptysis and anemia. In fact, 3 patients first presenting symptoms as adults have recently been studied at the West Roxbury Veterans Administration Hospital, and I can recall 2 cases in the past several months, which I have seen.

DR. JANEWAY. Do these lungs look different from those of a patient with mitral stenosis?

DR. VAWTER. The extensive fibrosis shown you by Dr. Sui Moto must be extremely rare, if indeed it ever ocurs, in mitral stenosis, unless of course there is some complicating cause for pulmonary fibrosis. In the boy discussed today, the pulmonary structure shows, I believe, no hint of the pulmonary vascular changes seen in mitral stenosis, although I have seen them in other patients of this age who have mitral stenosis.

DR. BIRD. I was just wondering whether calcification could contribute to or account for the persisting radiologic abnormalities in the boy Dr. Diamond described.

DR. VAWTER. In a few cases of diffuse calcification of pulmonary elastic tissue which have complicated vitamin D intoxication or hypercalcemia, the x-ray abnormality is sometimes quite minimal and when present has tended to be that of a diffuse ground glass, rather than a punctate density. I have not met anyone who has emphasized pulmonary calcinosis as a complication of hemosiderosis.

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Erratum. In the article, "The Protein Allowance in Infancy and Childhood" by Johnston and associates (J. PEDIAT. 59: 47, 1961) in Table IV on page 52, Formula 2, in the column listing weights, a decimal point has been omitted between the first and second figures for 4 subjects: Subject 14 gains 7.63 Gm./Kg./day
Subject 1 gains 6.01 Gm./Kg./day
Subject 12 gains 5.65 Gm./Kg./day
Subject 13 gains 2.85 Gm./Kg./day

COMMENTS ON CURRENT LITERATURE

Syphilis is still a problem

SYPHILIS remains at the present time a public health problem of major and increasing proportions. Public health reports[1] published late in 1960 carried the following statements: "cases of infectious syphilis have been increasing alarmingly since 1957. Moreover, there is no indication that the trend is changing. . . . We estimate the reservoir of untreated syphilitics today at 1,200,000 cases, and that the true annual incidence is 60,000 cases."[1] These reports emphasize the distinction between prevalence and incidence, the total number of cases, and the number of new infections. Effective control measures may bring about marked changes in the incidence which are not reflected rapidly in the prevalence of the disease. Reinfection and recurrence following inadequate treatment complicate the problem of public health control. Emphasis is placed likewise on the need for keeping a high index of suspicion regarding this so-called "dying disease," since a patient with early infectious syphilis may consult specialists about his various complaints without the true nature of the condition being apparent. This is true particularly in the adolescent "teen-age" patient. In one recorded instance the patient was seen over a period of time by 3 specialists before the true condition was suspected by a dermatologist.[2]

From the public health standpoint case finding is of great importance. Unfortunately patients with early syphilis are seldom acutely ill, and lesions of primary or even secondary syphilis are commonly insignificant and may be overlooked. It has been estimated from public health surveys that in only about 25 per cent of the new cases in a given community is a definite diagnosis made in the early infectious stage.[1] Every possible effort should be made to find early cases, a task which involves more lay education stressing the mild character of the early manifestations of the disease and urging the need for medical consultation. Physicians, including those caring for children, must be constantly alert for early signs of this infection. In a recent discussion concerning the problem of diagnosis Sciple, Montgomery, and Knox[2] describe the case of a schoolboy, 14 years of age, who was referred to the Venereal Disease Clinic because of a "positive" routine serologic test. Physical examination had been negative, and a tentative diagnosis of biologic false positive reaction was made by the referring physician. The patient had been attended by this referring physician for a persistent, nonspecific upper respiratory infection with pharyngitis and sore throat. All information relative to syphilis was negative, and the patient denied exposure of any kind. Serum serologic tests for syphilis at the referral clinic were reported as weakly reactive (VDRL), reactive (Kolmer), and nonreactive (Kahn). The cerebrospinal fluid Kolmer test was reported as nonreactive. Venereal Disease Research Laboratory tests of serum from the mother, father, and 10 siblings were

all nonreactive, as were cerebrospinal fluid examinations of the parents. On further interrogation statements made by both parents revealed a suggestive history, and both father and mother admitted infection and treatment before the birth of this son 14 years before. The more sensitive treponemal antibody tests performed after this information was obtained showed the mother's serum and the son's reactive, and the father's weakly reactive. These results together with the family history indicated congenital syphilis in the son. The limited therapy received by the mother during pregnancy was sufficient apparently for her "serologic cure," but was inadequate for her fetus. In view of such experiences the authors emphasize the justifiability of persistence in pursuing the history, both individual and family, and state that, in spite of the real problem of biologic false positive reactions, "a positive serologic test for syphilis should suggest a diagnosis of syphilis until proved otherwise."[3] This policy of high suspicion index was emphasized somewhat earlier by others, notably Perry, Kierland, and Màgath[4] in 1956.

In the case of congenital syphilis the infection is acquired in utero from the mother after about the fifth month of pregnancy.[5] The term prenatal syphilis has been suggested as more accurate than congenital syphilis. Pregnancy occurring in women with recent infection results almost invariably in miscarriage, stillbirth, or in congenital syphilis in the child. Women who become pregnant some years after infection give birth to normal children, as a rule. If syphilis can be recognized early in pregnancy and treated adequately, prenatal infection can be prevented in the majority of cases. The most effective case finding procedure for this group consists in the routine blood testing of all pregnant women.

Recognition of prenatal syphilis in the living infant born of a syphilitic mother presents a problem, since clinical evidence may not be apparent at birth. Diagnosis depends largely upon tests of cord blood; likewise, a study of the placenta and dark-field examination of tissue scrapings from the umbilical vein have been recommended.[6] If the mother's serology is positive at the time of delivery, a passive transfer of reagins to the infant may have occurred in the absence of fetal infection.[7] Relatively few of these infants show manifestations of infection, and many revert to negative in from 1 to 5 months with no treatment.[8] However, a rising or persisting high titer justifies the diagnosis and the institution of treatment. Symptoms and signs of prenatal infection usually appear within the first 3 to 4 weeks of life, and characteristically include a maculopapular rash, snuffles, mucocutaneous fissures, enlarged liver and spleen, and hoarse, aphonic cry. Bullous lesions on the palms and soles are considered pathognomonic. Bone manifestations include osteochondritis, chondroepiphysitis, and osteomyelitis; pseudoparalysis may be a feature. Osteitis results in the characteristic saddle deformity of the nose. Later manifestations comprise mainly the so-called developmental stigmas: Hutchinsonian teeth, mulberry molars, enlarged frontal bossae, sabre shins, and scaphoid scapulae. Inflammatory lesions are of importance: interstitial keratitis, eighth nerve deafness, hydrarthrosis, etc. The occurrence of neurosyphilis constitutes one of the most serious manifestations leading to taboparesis and mental retardation.

Studies in the field of serodiagnosis conducted within the past few years have shown the treponemal tests (*Treponema pallidum* immobilization, *Treponema pallidum* complement fixation, Reiter protein complement fixation, and the fluorescent Treponemal antibody tests) more reliable (e.g., more specific) indicators of the presence of asymptomatic syphilis than the reagin tests (Wassermann, Kolmer, VDRL, etc.). Of these more specific procedures, the Reiter protein complement fixation test is gaining wide acceptance because of its good duplicability and easy performance.[9]

Since the first use of penicillin in 1943,[10] the effectiveness of this therapy alone, in adequate dosage, has been proved by the absence of serologic and clinical recurrence in cases of early syphilis, as well as by its

efficacy in the prevention of prenatal infection.[11] Experience with penicillin therapy emphasizes maintenance of continuous effective blood levels for from 7 to 15 days. The form of penicillin employed appears to be an individual matter: water-soluble penicillin G at frequent intervals; twice-weekly administration of procaine pencillin with 2 per cent aluminum monostearate (PAM); or a single injection of long-acting benzathine penicillin G. The need for convenient or ambulatory management has made PAM or benzathine penicillin G the therapy of choice. In infants and young children individualization is the rule. In hospitalized cases aqueous penicillin by frequent injection has been recommended to be continued over a period of 15 days, the total dose being 100,000 units to 400,000 units per kilogram of body weight. This dose may be administered in 120 individual injections 3 hours apart. There appears to be little advantage in total doses higher than 100,000 units per kilogram of body weight.

Penicillin readily permeates the placenta from about the tenth week of gestation. This has been shown by studies in experimental animals by the determination of penicillin in fetal tissues and by healing syphilitic lesions in newborn infants following antepartum therapy. While a cure may not be effected in the mother, she may give birth to a normal infant, especially when dosage of the antibiotic is high. The infrequent failures following penicillin treatment usually result from inadequate treatment or from a fetus severely damaged before therapy was instituted, as when infection is discovered late in pregnancy, beyond about 30 weeks. For patients hospitalized during pregnancy a therapeutic regimen of aqueous penicillin is recommended, 40,000 to 50,000 Oxford units every 2 to 3 hours, with a total of 2.4 million units or more over a course of 7 to 12 days. In ambulatory patients, procaine penicillin G has been used successfully, 600,000 units once daily for from 8 to 10 days, with a total of 4.8 to 6.0 million units of penicillin.

Sensitivity to penicillin may pose a problem,[12] and in some instances the enzyme penicillinase has been used as a therapeutic agent in the management of reactions.[12] In experimental animals and in human subjects, the parenteral administration of this enzyme results in the rapid disappearance of detectable circulating penicillin. In this connection a recent report by Greaves[13] is of interest. An objective clinical analysis of the effect of penicillinase was made by determining the degree of spirochetal protection when penicillin and penicillinase were administered concurrently to syphilitic patients with dark-field positive lesions. The author reasoned that "if penicillinase destroys all penicillin, then *Treponema pallidum* will continue to survive in their chancres or moist eruptions."[13] Patients with dark-field lesions were divided into categories, one group receiving 600,000 units of penicillin in aluminum monostearate intramuscularly, preceded by 800,000 units of penicillinase parenterally; the corresponding control group received the same dosage of penicillin alone. Hourly dark-field examinations and penicillin blood levels were carried out. *T. pallidum* disappeared from the lesions of both groups of patients with no statistical differences between the two. Penicillin blood levels dropped below the detectable range in the test group, and in some instances failed to rise when penicillin alone was resumed. Nevertheless, organisms disappeared from the lesions and healing occurred. Many patients showed serologic improvement. With respect to penicillinase as a therapeutic agent in penicillin sensitivity reactions, the author concludes that this enzyme cannot be considered adequate therapy for life-threatening reactions, since penicillin may be suppressed but not neutralized.

While the treponemicidal properties of erythromycin have been known for about 10 years, adequate clinical information concerning its oral use has been available only recently. Montgomery, Knox, and their associates[14] have been interested in the possibilities of this antibiotic for a number of years and have published recently a report concerned with the use of erythromycin

stearate and erythromycin propionate in the treatment of early syphilis.[14] Since large doses are required for effective antisyphilitic treatment, the authors sought minimal effective dose levels. In a study of 148 darkfield positive patients, the use of 20 Gm. of either erythromycin stearate or erythromycin propionate distributed over a 10-day period appeared adequate for the treatment of early syphilis. In some instances erythromycin propionate, 500 mg., 3 times daily for 10 days (15 Gm.), was effective.

A recent composite report on "Today's VD Control Problem"[15] published in booklet form contains valuable information and urges again that the practicing physician be on the alert for early signs of infection and that he report suspected cases immediately, especially among young people, since "there continued to be outbreaks of syphilis in 25 states and 18 cities during the present year."[15] Recent statistics compiled in one urban area (Houston, Texas) indicate that in the period, January 1960 through February 1961, 22.4 per cent of the cases of primary and secondary syphilis occurred in the 15 to 19 year age group, and 39.3 per cent in the 20 to 24 year age group.[16]

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Books

Clinical Disturbances of Renal Function. Abraham G. White, M.D., Philadelphia, 1961, W. B. Saunders Company, 468 pages. Price $10.50.

As the "Preface" points out, this book is meant for the practicing physician confronted with a patient whose kidneys are not functioning normally. The author is a man of very considerable clinical and laboratory experience in this field, and well qualified to write on this subject.

The first several chapters of this book are devoted to primary renal diseases and include an excellent chapter on hypertension and its relation to the kidney. In the remainder of the book are discussed the effects on renal function of surgery, pregnancy, urologic problems, congestive heart failure, liver disease, hormones, genetic influences, and neuropsychiatric forces.

One of the 2 major defects of this book consists in the author's tendency to make categorical statements on certain controversial matters. For example, he states that extrapharyngeal streptococcal infections are *not* followed by renal disease. Again, he says that sodium-containing alkali should not be administered to correct the metabolic acidosis of acute renal failure. On the whole this is sound advice, but there are instances of severe depression of the blood pH when many competent physicians would administer alkali.

The second shortcoming, from the pediatrician's point of view, lies in the failure to relate the discussion specifically to diseases as seen in children. Dosage schedules for infants and children are rarely given, and descriptions of disease occasionally differ sharply from what is seen in pediatric subjects.

There are, however, so few good books on the practical aspects of renal dysfunction that the reader must not be too critical. The pediatrician will profit from a reading of this book by gaining an appreciation of the logical physiologic approach to kidney disease and function.

DANIEL S. FLEISHER, M.D.

Congenital Malformations of the Heart. Helen B. Taussig, second edition, Cambridge, 1960, published for the Commonwealth Fund by Harvard University Press. *Vol. I, General Considerations,* $4.75; *Vol. II, Specific Malformations,* $17.50.

Any physician interested in this subject must give the second edition of this work careful study. This author's detailed clinical approach, although often repetitious, is unique and valuable; the completeness of each section simplifies the reader's task.

Although this work is not a textbook on electrocardiography or radiologic cardiography study, it demonstrates clearly how the clinician uses these tools in clinical evaluation. In the new "Visual Index" the main clinical, roentgen and electrocardiographic characteristics of each malformation are shown simply and concisely.

Representing as it does the lifetime experience of a pioneer in this field—an experience unequaled as to volume of material—the second edition of this book with its many additions and corrections, has great significance for the student of this very complicated subject.

C. ROBERT E. WELLS, M.D.

Correspondence

Infrapulmonary pleural effusion

To the Editor:

In the article by Hotzel and Fawcitt[1] reference is made to a paper which I gave at the Ninth International Congress of Pediatrics in Montreal, 1959. Since no official transcript of the transactions of the Congress was published, it is understandable that this paper has been misquoted, particularly in the "Editor's Note" appended to the article by Hotzel and Fawcitt. In view of the interest and importance that is attached to accurate recognition of pleural fluid in inflammatory and noninflammatory conditions, I beg your leave to offer some comments.

In 1959 we published[2] our findings from a review of 49 patients who had radiologic examinations of the chest made during the active stage of the nephrotic syndrome and showed that approximately 75 per cent of them had "infrapulmonary pleural effusion" (I.P.P.E.). This term referred to pleural fluid collected between the base of the lung and diaphragm and having a concave upper surface readily mistaken for the normal or elevated diaphragmatic contour (Fig. 1). The previous literature had contained case reports of only 8 patients with nephrosis and pleural fluid. Our review indicated that this association was the rule rather than the exception (since 1959 further experience has indicated that the percentage of patients with active nephrosis having demonstrable collections of pleural fluid is closer to 100 than 75). It was pointed out that chest films of patients with nephrosis have a rather typical and recognizable appearance, including soft tissue edema, unilateral or bilateral I.P.P.E., and low position of the heart relative to the bases of the lungs, but that the same appearance may be expected in patients having hypoproteinemia from other causes.

It should not be assumed, however, that infrapulmonary localization of pleural fluid only occurs in nephrosis or even in hypoproteinemia. Although there is a remarkable paucity of available information about the physical properties of normal or abnormal pleural fluid, including its surface tension and specific gravity, the phenomenon of infrapulmonary pleural effusion is

Fig. 1*A*. From Dunbar and Favreau: J. Canad. A. Radiologists 10: 24, 1959.

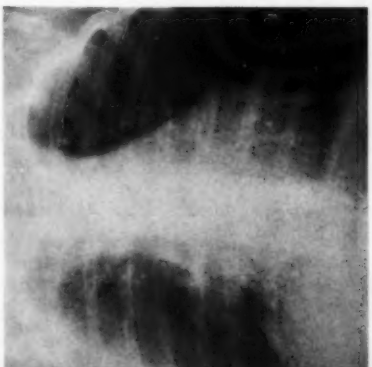

Fig. 1*B*. Lateral view.

Fig. 2*A*.

Fig. 2*B*. Lateral view.

by no means restricted to noninflammatory conditions. Fig. 2A shows the AP supine chest film of a patient with empyema, and Fig. 2B the same patient in the left-side-down ("left lateral decubitus") position, demonstrating the displacement of the fluid in the pleural space.

Far from suggesting that the phenomenon of I.P.P.E. is restricted to noninflammatory conditions, we believe that it is of the utmost importance to recognize that it may occur in virtually any condition in which the fluid is freely mobile. Particularly in empyema of infancy and early childhood, early diagnosis and treatment may depend on recognition of this phenomenon. The excellent article by Hessen in 1951[3] had clearly and thoroughly documented the roentgen findings of I.P.P.E. Our studies have added one further sign to aid in the recognition of I.P.P.E. but in particular have shown that when one is aware of this phenomenon, pleural fluid is almost always demonstrable in patients with active nephrosis.

It is really more important to recognize and demonstrate pleural fluid in other conditions, particularly inflammation, when early diagnosis and treatment are essential.

J. S. DUNBAR, M.D.

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Gamma globulin dosage

To the Editor:

The dosage of gamma globulin recommended for adults for the *prevention* of hepatitis with jaundice in the 1961 edition of the *Redbook* of the American Academy of Pediatrics has been questioned by Drs. Gellis and McComb in their communication "Help Conserve Gamma Globulin." The Committee on Control of Infectious Diseases which is charged with the revision of the *Redbook* realizes that the optimum dose of gamma globulin for adults is not known. Although there is good evidence based on well-controlled studies that 0.01 ml. per pound is effective in preventing jaundice in children, comparable evidence is lacking in adults.

Recommended dosages by various investigators and authoritative groups vary from 0.01 ml. per pound to 0.06 ml. per pound. The *Armed Forces Technical Bulletin, Medicine,* states that a dose of 0.05 ml. per pound is recommended. This dosage has been reaffirmed in March, 1961, and again in June, 1961, by the Hepatitis Subgroup

of the Commission on Viral Infection of the Armed Forces Epidemiological Board. The studies of Krugman and associates (J.A.M.A. 174:823, 1960), which are the most satisfactorily controlled, indicate that the effective dose for prevention of hepatitis with jaundice in adults may be as high as 0.06 ml. per pound. The Committee recognizes that a single lot of gamma globulin was used in these studies. In another recent Letter to the Editor (J.A.M.A. 176:560, 1961) Dr. Joseph Stokes, Jr. stated that an "educated guess" regarding the dosage of gamma globulin for adults exposed to endemic infectious hepatitis would be 0.06 ml. per pound every 5 or 6 months.

This evidence, admittedly inconclusive, influenced the Committee to advocate a larger dose for adults than for children. Another consideration in recommending a higher dose for adults is the well-known fact that hepatitis can be much more severe in adults than in children.

The Committee on Control of Infectious Diseases makes its recommendations on the basis of *available* scientific evidence. It is hoped that further investigations of the effectiveness of various dosages of gamma globulin in preventing or modifying hepatitis in adults may provide information on which more definite recommendations to aid the physician consulted about parents or other exposed adults can be based.

AMERICAN ACADEMY OF PEDIATRICS COMMITTEE ON CONTROL OF INFECTIOUS DISEASES

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A living image of my beliefs

To the Editor:

The following note was sent to us by Dr. Harry H. Gordon of Baltimore, who observes, "In these days when some people seem to be confused about the pleasures obtained from the practice of pediatrics, the enclosed short theme written by a high school student about a practitioner of pediatrics is heartening."

A Living Image of My Beliefs

When I think of the people with whom I am associated, I find many who are worthy of my admiration, more who deserve my respect, and a few who have earned my love. There is no need to dwell on the depth of devotion which exists between my family and me; for this is a truth with which I have lived securely all of my life. But those whom I love most dearly do not necessarily personify the ideals which are closest to my heart. When I think of the life ahead of me and of what use I may make of it, the image of one man arises before me.

He has known me since the second day of my life. He has healed, guarded, cautioned, and counseled me. He is my devoted friend and physician, Dr. X. For as long as I have been able to understand and appreciate all that is fine, I have admired, and respected, and loved him. He is an outstanding medical man—deeply devoted by day and by night to the care of his charges. When it occurred to him that, in keeping with his specialty of Pediatrics, he would be losing contact with his patients at their very crucial adolescent years, he added to his already monumental work-load by continuing to care for his "babies" now fast approaching young adulthood.

Doctor X is not only an eminent physician. He is a humanitarian and a patriot of the world. He is a champion of the oppressed, and a staunch supporter of liberty and learning.

Notwithstanding the wide scope of his work and the demands on him, Doctor X has time to be a down-to-earth, pleasure-loving, easy-to-talk-with friend and advisor. He isn't too busy or too aloof to invite his 15-year-old patient to a golf match. He makes the most of every opportunity to discuss the challenges which face his young people. He advises on education and careers. He praises success; he stimulates the inactive; he understands and often forgives shortcomings. Is there any wonder then, that since I have found no higher aim, no nobler purpose, no beliefs more closely related to mine, that I should sincerely hope to follow in the footsteps of my friend and physician, Doctor X?

The role of lysolecithin and cholesterol in icterus neonatorum

To the Editor:

Gotlieb, Pesach, and Rimon in the paper "The role of lysolecithin and cholesterol in icterus neonatorum" (J. PEDIAT. **58:** 828, 1961) have not defined the latter term. If they refer to physiologic icterus of the newborn, it would be necessary to rule out the known causes of neonatal jaundice before making this diagnosis of exclusion. These data are essential to their conclusion that "The enzymatic formation of lysolecithin is suggested as a causative factor of the hemolysis." If their icteric group is heterogeneous and contains unsuspected cases of blood group incompatibility (with mild hemolysis), one may be able to conclude that the finding of lysolecithin is the result rather than the cause of hemolysis in the neonate.

RICHARD H. RAPKIN, M.D.

Reply

I am sorry that it was not clearly stated in our paper that the investigation covered only clear-cut cases of icterus neonatorum or physiologic icterus of the newborn, as far as could be ascertained.

All cases of erythroblastosis fetalis, ABO incompatibility, or hemolysis due to other known causes were excluded a priori from this study.

Out of the 18 cases in the icteric group, 12 having the highest levels of bilirubin on the fourth day were more carefully studied but no blood incompatibility could be detected.

We believe, therefore, that our groups of infants were quite homogeneous, as far as could be ascertained.

ABRAHAM GOTLIEB, M.D.

*News and notes**

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The twenty-ninth annual **Benjamin Knox Rachford Lectureship** will be held in Cincinnati, in the Auditorium of the Children's Hospital Clinic and Research Building, on Oct. 10 and 11, 1961, at 8:30 P.M. Dr. Paul Hamilton Wood, Director of the Institute of Cardiology of London, will speak on "The Natural History of Congenital Septal Defects" and in the first lecture will discuss atrial septal defects; in the second, ventricular septal defects.

Applications for the **Residency in Pediatric Oncology,** July 1, 1962, to June 30, 1963, at Memorial Hospital for Cancer and Allied Diseases in New York City will be received up to Nov. 15, 1961. This residency is approved for partial credit by the American Board of Pediatrics and is limited to the neoplastic diseases of children. Salary is $3,600 annually plus maintenance. For application forms and information write: Harold W. Dargeon, M.D., Chairman, Department of Pediatrics, Memorial Hospital for Cancer and Allied Diseases, 444 East 68th Street, New York 21, N. Y.

Fellowships in pediatric hematology and oncology are offered by the Children's Hospital of Los Angeles, Department of Pediatrics, University of Southern California, to begin July 1, 1962. The program is designed to prepare qualified individuals for careers in academic medicine and research. Some previous experience in hematology or a preclinical science related to hematology is desirable. Applicants should have completed pediatric training acceptable for certification by the American Board of Pediatrics; preference will be given to applicants interested in training for a period of 2 or more years, but applications for a single year will be considered. Stipends provide $4,000 per annum or more. For further information write Denman Hammond, M.D., Children's Hospital of Los Angeles, 4570 Sunset Boulevard, Los Angeles 27, Calif.

The **University of Kentucky Medical Center** announces the inauguration of a full-time teaching program in pediatrics in July, 1962, to include: (1) pediatric internships, consisting of rotation through the outpatient department, nurseries for newborn infants, and inpatient

services; (2) two-year residencies combining outpatient and inpatient experience with periods in subspecialties and laboratories; and (3) fellowships in the pediatric subspecialties, with the possibility of a combined fellowship in the various specialties for qualified persons. Communications should be addressed to Dr. John H. Githens, University of Kentucky Medical Center, Lexington, Ky.

The **University of Pittsburgh School of Medicine** announces a training program in **academic pediatrics,** combining instruction in basic science with an opportunity for specialization in the field of major interest. Ordinarily a 2-year service is advised, but 1-year fellowships are available. Stipends begin at $6,000 with additional allowances for dependents; applicants must have completed the residency requirements of the American Board of Pediatrics. Address inquiries to Richard L. Day, M.D., Professor of Pediatrics, Children's Hospital of Pittsburgh, 125 De Soto Street, Pittsburgh 13, Pa.

The **Children's Hospital "Aghia Sophia,"** Athens, Greece, which offers a postgraduate training program in pediatrics, requests assistance in establishing a medical library. Back volumes of the JOURNAL would be especially appreciated. Donations should be sent to The Library, Children's Hospital "Aghia Sophia," Athens, Greece.

EDITOR'S COLUMN

Jaundice in the African neonate

IN MOST parts of Africa, as in other tropical regions, too little is known of the pattern of disease in the newborn period. Infants born in often quite distant villages or rural areas are unlikely to be seen by a modern doctor, while, with the intense pressure on maternity accommodations, mothers remain in the hospital only 2 or 3 days after delivery, so that observation of the neonate cannot easily be continued after this. Knowledge concerning the incidence of neonatal jaundice in African children is very incomplete, partly because of the great difficulty of its recognition in darker-skinned infants, and partly because of their rapid discharge from the hospital. However, there is good evidence to suggest that the etiological emphasis is different from that in the temperate zone.

In tropical African circumstances (and the same probably applies in most technically developing countries with low levels of hygiene) neonatal septicemia, arising from umbilical infection, easily heads the list.[1, 2] In addition, especially in towns and seaports, congenital syphilis always requires diagnostic consideration, although in many parts of Africa it is puzzlingly uncommon.

The situation with regard to Rh isoimmunization is intriguing and only partly explained. Scanty reports of uncommon cases have appeared in the literature from South Africa,[3] Uganda,[4] and Kenya[5] and notes proclaiming its rarity from elsewhere, as in Lagos[1] and Dakar.[6]

It has been known for years that the incidence of Rh negative persons is less among African populations (ranging, for example, from about 2 to 8 in Kenya) as compared with Caucasians. However, as remarked by Jacob, Macdougall, and Davies[5] in Kenya, assuming an average of 4 per cent Rh negative persons in this region, a calculated 0.3 per cent of all newborn infants might be expected to have hemolytic disease due to Rh sensitization.

Then is the apparent uncommonness of this condition due (as with so many hastily formed concepts concerning tropical pediatrics) to lack of recognition in the short-staffed, meager, medical facilities, preoccupied with grosser (and expected) disease problems, as, for example, tetanus neonatorum and septicemia? Or, is there some unknown protective mechanism at work? Only epidemiologic studies can give the answer. It has been rightly emphasized that it is a sound rule in pediatrics in Africa that the expectation of disease should be based on generally accepted principles, at least until the accumulation of facts can prove otherwise.[5] However, the possibility of some protective mechanism may be partly correct, as suggested by Scott and his associates[7] who found an incidence of only 0.01 per cent of

hemolytic disease due to Rh sensitization in a total of 11,217 births among American Negroes.

Theoretical causes of jaundice in the newborn infant in Africa include sickle cell anemia and other hemoglobinopathies. The first was considered in the very recent "early" days of awakening awareness of the widespreadness of sickle cell disease in Africa. (The amazing increase in knowledge concerning the hemoglobinopathies is stressed by the fact that the first cases of sickle cell anemia were reported from Nigeria as late as 1952.[8]) Subsequent work, however, has shown that the persistence of fetal hemoglobin in the baby postpones the attainment of full sickle cell status until the second semester of life, so that hemolysis from this cause would not be expected in the newborn infant. However, the report by Boyo and Hendrickse[9] of a new "fast" hemoglobin in newborn Nigerians introduces a new factor.

Recent work on enzyme defects in red blood corpuscles promises to be of importance in relation to the occasional markedly jaundiced "normal" African neonate. In particular, there is evidence that deficiency of glucose-6-phosphate dehydrogenase may be common in African communities, and (almost inevitably) it has been suggested that the harmful effect of having cells which are abnormally easily hemolyzable may be counterbalanced by partial protection against malignant tertian malaria.

In Ibadan, Nigeria, Gilles and colleagues[10] have found about 20 per cent of Yorubas possess this deficiency in their erythrocytes and have shown that hemoglobinuria due to drug sensitivity occurs in adults.[11] In addition, Weatherall[12] has described 3 Malay neonates seen in Singapore whose hemolytic condition was unconnected with Rh or ABO incompatibility, but was considered to be related to glucose-6-phosphate dehydrogenase deficiency. The possibility of a similar condition being found in African newborn infants undoubtedly requires investigation.

D. B. JELLIFFE, M.D.

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